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14. ABSTRACT (maximum 200 words)

The objective was to assess the neurotoxicity of ammonium perchlorate as it relates to the development of the motor system. Ammonium perchlorate is is used in solid rocket propellant systems, and has been found in ground water at sites where this propellant is manufactured and stored. It readily dissociates in water and produces perchlorate ion that displaces the iodide (I^-) anion and disrupts thyroid activity. The thyroid becomes underactive (hypothyroidism), leading to reduced levels of thyroid hormones triiodothyronine (T3) and thyroxine (T4). There is some evidence to suggest thyroid hormones play an important role in normal brain development, including areas of the brain related to motor activity. Thus, a neurobehavioral test for spontaneous locomotor activity was employed to detect developmental abnormalities within the brain systems related to gross motor movement.

Female rats were treated for two weeks prior to gestation through post-natal day (PND) 10 with one of 5 doses of ammonium perchlorate in their drinking water (0, 0.1, 1.0, 3.0, or 10.0 mg/kg/day). One male and female rat pup were randomly selected from each litter for testing of general locomotor activity at three preweanling ages – PNDs 14, 18, and 22. Pups were individually tested in automated Opto-Varimex Activity boxes where 9 different measures of activity were recorded for 90 consecutive minutes on each test day. Data was divided into 9, 10-minute blocks, and was analyzed separately for each of the 9 dependent variables using a repeated measures ANOVA. The main effect for drug dose was not significant for any of the 9 measures, and there were no reliable interactions for treatment. Statistically reliable results were found for expected effects, such as changes in overall activity at different ages, and reduced activity from the start of a given test session to the end of the session. Overall, the results suggest there was not a significant change in general locomotor activity due to pre- and neonatal ammonium perchlorate.

15. SUBJECT TERMS
ammonium perchlorate, motor activity, habituation, neurobehavior

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A NEURODEVELOPMENTAL STUDY OF THE EFFECTS OF ORAL AMMONIUM PERCHLORATE EXPOSURE ON THE MOTOR ACTIVITY OF PRE-WEANLING RAT PUPS

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Report No. TOXDET-00-03 supported by Navy work unit #10004. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government. Approved for public release, distribution unlimited. The research reported herein were conducted according to the principles set forth in the "guide for the Care and Use of Laboratory Animals," Institute of Laboratory animal Resources, National Research Council, DHHS, Publication No. (NIH) 86-23 (1996). The activities were approved by the Wright-Patterson Air Force Base Institutional Animal Care and Use Committee as written in protocol #F-WA-2000-0038, and by the Wright State University Laboratory Animal Care and Use Committee as written in protocol #433.

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Preface

This report summarizes research conducted at the Naval Health Research Center Detachment (Toxicology) under Navy work unit #10004 through sponsorship from AFRL/HEST. The work was completed between February 2000 and June 2000.

The authors would like to acknowledge Dr. Glenn Ritchie of Geo-Centers and Dr. Terrence Deak of ManTech/Geo-Centers Joint Venture for their comments and suggestions related to this manuscript. The following people are recognized for their technical assistance: Sue Prues and Shawn McInturf of Geo-Centers, and Kimberly Rice, Claudine Volkart, Stacey Elmore, Rachel Salacinski, and Fred McDougal of NHRC/TD. The authors also thank J. Eric Eldridge of AFRL/HEST and Rebecca Clewell of Geo-Centers for completing ion chromatography analyses. A very special appreciation is extended to Dr. John Tisak, of Bowling Green State University, for his timely statistical consultation.

Summary

The objective of this work unit was to assess the neurodevelopmental consequences of ammonium perchlorate as it relates to the mammalian motor system. Ammonium perchlorate is a powerful oxidizer used in solid rocket propellant systems, and has been found to be a ground water contaminant at sites where this propellant is manufactured and stored. The primary concern with ammonium perchlorate is that it readily dissociates in water and produces the perchlorate ion that disrupts thyroid activity as it displaces the iodide (I^-) anion. As a result the thyroid becomes underactive, a condition of hypothyroidism, leading to a subsequent reduction in the levels of thyroid hormones such as triiodothyronine (T_3) and thyroxine (T_4). The problem addressed in this research was the neurodevelopmental effect of hypothyroidism since there is some evidence to suggest thyroid hormones play an important role in normal brain development, including areas of the brain related to motor activity. Specifically, a neurobehavioral test for spontaneous locomotor activity was employed to detect developmental abnormalities within the brain correlates of gross motor movement.

To address the research question, female rats were treated for two weeks prior to gestation through to post-natal day (PND) 10 with one of 5 doses of ammonium perchlorate in their drinking water. One male and female rat pup were randomly selected from each litter for testing of general locomotor activity at three preweanling ages – PNDs 14, 18, and 22. Pups were individually tested in automated Opto-Varimex Activity boxes where 9 different measures of activity were recorded for 90 consecutive minutes on each test day. Data were analyzed in 9, 10-minute blocks using a repeated measures ANOVA.

The main effect for drug dose was not significant for any of the 9 dependent variables, and there were no reliable interactions for treatment. The statistically reliable results indicated expected effects, such as increased activity when the animals were older, and reduced activity from the start of a given test session to the end of the session. Overall, the results suggest there was not a significant change in general locomotor activity due to pre- and neonatal perchlorate exposure.

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A NEURODEVELOPMENTAL STUDY OF THE EFFECTS OF ORAL AMMONIUM PERCHLORATE EXPOSURE ON THE MOTOR ACTIVITY OF PRE-WEANLING RAT PUPS

Introduction

Neurobehavioral tests are regularly used to screen for anomalies in brain development and function. Performance is quantified, and scores can be used to infer the integrity of neural systems related to the specific task. These tests are particularly useful as animal models of human behavior in order to evaluate effects of potentially harmful substances. In the present research, a test for measuring spontaneous locomotor activity of rat pups was used to evaluate neurotoxic insults in a developing rat brain. The neurodevelopmental effects of exposure to a substance known to reduce thyroid function and subsequent production of critical thyroid hormones were assessed.

Normal functioning of the thyroid gland is part of a relatively simple feedback loop with the brain. Iodide (I^-) is crucial to the normal functioning of the thyroid, and is an essential ion for normal production of the thyroid hormone thyroxine (T_4). In turn, triiodothyronine (T_3) is derived from T_4 such that interference with T_4 production subsequently reduces levels of T_3. Production of T_4 is stimulated when low levels of T_3 in the brain signal increased release of thyroid stimulating hormone (TSH). If the neural message for increased T_4 synthesis is impeded, a condition of hypothyroidism, or goiter, results. This is the case when animals are exposed to the perchlorate anion. Perchlorate competitively inhibits accumulation of I^- in the thyroid gland. The absence of I^- halts production of T_4, thus stimulating the feedback cycle signalling for TSH. However, little or no T_4 is produced due to the displacement of I^- and hypothyroidism results. Due to these effects, perchlorate is used to treat Graves' disease, a condition of hyperthyroidism. However, uncontrolled exposure to perchlorate, such as through contaminated drinking water, is a health concern addressed in the present research.

Perchlorate is an anion that is easily dissociated from salts such as ammonium perchlorate or sodium perchlorate. It has been found contaminating ground water and soil particularly in the southwestern states of California and Nevada. These are sites of production and storage of ammonium perchlorate, a strong oxidizer used by the Department of Defense in propellant systems such as those found in rockets and munitions. Ammonium perchlorate is readily soluble in water, and the long-term stability of the perchlorate ion has been demonstrated (Tsui et al., 1998). Because of possible prolonged contamination in the drinking water supply, there is considerable potential for exposure in people working and living near facilities where the oxidant is manufactured and stored. One specific area of concern is the effect on processes controlling neurogenesis and synaptogenesis in developing fetuses of females exposed to perchlorate.

Research with pregnant female rats indicates increased thyroid size in both the dams and pups following maternal perchlorate exposure. As expected, enlargement of the thyroid gland directly correlated with reduced I^- uptake in the dams, fetuses, and nursing pups (Brown-Grant, 1966; Brown-Grant and Sherwood, 1971). The most notable changes in I^- uptake in the fetuses were found in the final few days of gestation, the stage when the thyroid becomes active in the developing rat fetus (Sztanyik and Turai, 1988). In the case of nursing pups, the evidence suggests perchlorate is not transferred through the dam's milk, rather, the amount of I^- in the milk is significantly reduced (Brown-Grant and Sherwood, 1971; Zeghal et al., 1992). This low level of available I^- has been correlated with decreased levels of both T_3 and T_4 in pups treated pre- and neonatally with perchlorate (Golstein et al., 1988), and with a higher concentration of T_3 receptors found in the brain of hypothyroid pups on postnatal day (PND)14 (Ishiguro et al., 1980). Such evidence suggests that any T_3 and/or T_4-dependent processes of normal brain development would likely be delayed or otherwise abnormal in the pups of dams treated with perchlorate.

Previous research has suggested that the cerebellum is exquisitely sensitive to the deleterious consequences of toxic chemicals (Fonnum & Lock, 2000). Furthermore, development of the cerebellum is also vulnerable to the effects of early deficiencies in thyroid hormones. For example, reduced myelin formation has been reported in the cerebellum of hypothyroid rat pups

as compared to controls (McIntosh et al., 1981; Pasquini and Adamo., 1994). Myelin surrounds the neuronal extensions and is critical for proper electrical conductivity and communication amongst brain cells. Also, reduced cerebellar weight (Walker et al., 1989), and smaller cell size of cerebellar neurons (McIntosh et al., 1981) have been reported for hypothyroid rat pups. The smaller cell size could be indicative of delayed or disrupted cell differentiation, an effect further evidenced in elevated neural density and smaller average neuron size in pups from dams fed a severely iodine deficient diet (Li et al., 1986). These changes suggest that early exposure to perchlorate, and the effects it has on thyroid hormones, may result in abnormal cerebellar development. Given the critical role of the cerebellum in general motor activity and coordination (Middleton and Strick, 2000; Armstrong et al., 1997), an early insult at this level may be predicted to manifest itself in atypical patterns of spontaneous locomotor responding.

In summary, data from previous research suggests hypothyroidism interferes with normal brain development, including changes in a brain area critical for the integrity of motor coordination (Pasquini and Adamo, 1994; Chan and Kilby, 2000). If there are abnormalities during maturation of locomotor control systems in the brain, it is likely that they would be revealed in tests of neurobehavior related to those systems. In the present investigation, the test of open field motor activity was used to evaluate pups from dams treated with different doses of ammonium perchlorate in their drinking water. Overall activity of an animal in an open field can be used to assess the integrity of brain systems related to gross motor movement, general exploratory activity, and habituation to a new environment. The open field measure can quantify animal activity ranging from totally unresponsive to hyperactive, and thus is ideal for assessing both increases and decreases in overall locomotor activity.

Methods

Animals

One hundred ten adult virgin female Sprague Dawley (Crl:CD BR VAF/+) rats and 37 adult male Sprague Dawley rats used in this study were obtained commercially from Charles River Laboratories, Wilmington, MA. Animals were housed in plastic shoe box-like cages lined with

Sani-Chips absorbent bedding, and maintained on a 12-hour diurnal cycle. Food (Teklad Certified Rodent Diet) and water (Type 1 water purified to >18.0 megaohm-cm resistivity) were available *ad libitum*. Immediately following arrival, all animals were housed in quarantine for 2 weeks, during which time the female rats and their water bottles were weighed daily between 0730 and 1200. No body weights or water bottle weights were taken for the male breeders.

Following the quarantine period, dams were randomly assigned to 1 of 5 dosing groups. Ammonium perchlorate was dissolved in their drinking water at specific concentrations so that dams received doses of 0, 0.1, 1.0, 3.0, or 10.0 mg/kg/day. Dosing concentrations were monitored and confirmed on a regular basis using ion chromatography. Dam body weights and amount of water drank were monitored on a daily basis (excluding breeding days) to ensure close approximations of the target doses. Dams were dosed for two weeks prior to mating with the breeder males, and through to PND10. For breeding purposes, individual males were randomly paired with each female and both were placed in a standard home cage with breeding grids placed on the bottom in the place of bedding. Every morning the cages were surveyed for vaginal plugs. If one was found, the date was recorded as gestation day (GD) 1, the male was returned to his home cage and the female was placed into a new cage with clean bedding. If no plug was discovered, the male and dam were left together until a vaginal plug was found. If mating was unsuccessful for more than 5 days, the dam was eliminated from the study and was euthanized.

Once a vaginal plug was confirmed, dams were weighed 3-4 days per week, at a rate of approximately every other day. Daily monitoring of water intake was continued throughout the gestation period. As the expected parturition dates neared, animals were checked 2-3 times daily for birth of pups. PND1 was counted as the day when the first pup was observed in the cage. Dams were not weighed on PND1, but were weighed 3-4 days per week until PND10 beginning PND2 or PND3. All pups within a litter were weighed on PND5, when the litters were culled to 8 pups of 4 males and 4 females, or as close as possible to this combination. Pups and dams from any litters with less than 8 pups were eliminated from the study and euthanized. During

PND5-PND10, pups' tails were tattooed in a dot pattern used to separate males from females, and identify individual pups within a litter.

Opto-Varimex Auto-Track System

The Opto-Varimex activity meters, purchased from Columbus Instruments, Columbus, OH, are 17" x 17" Plexiglas open fields with infrared photocells placed 2.4 cm apart along the perimeter of the fields. There are two different levels of photocells to detect both horizontal and vertical movements, as well as differentiate small (stereotypic) from large movements. In all, 9 different measures of motor activity are automatically recorded: frequency and time of ambulatory movements, frequency and time of stereotypic movements, frequency of movements in the horizontal plane, distance traveled in the horizontal plane, frequency of rears, total number of horizontal movements made while in the rearing position (vertical plane movements), and time spent resting.

Motor Activity Testing

On PND14, one male and one female were randomly selected from each litter to be used in motor activity testing. These same animals were tested on PNDs 14, 18, and 22. On each test day, pups were placed in individual transport cages that were similar to their home cages and lined with fresh bedding, for moving the animals to the testing room. Upon arrival at the test room, they were left in the transport cages for 5-7 minutes to habituate to the low red lighting and white noise (70dB). Following habituation, the pups were individually tested for 90 minutes in automated Opto-Varimex animal activity meters. Throughout the testing session, the only illumination was red light from 25W bulbs placed above pairs of testing boxes. To start the 90-minute test session, each pup was placed directly in the middle of the open field. Immediately after the pups were placed in their respective fields the Auto-Track data recording system was started and the pups were left undisturbed throughout the 90-minute test session. Upon completion, the pups were removed from the open fields, placed in their transport cages, and returned to their home cages. Between each test the open fields were washed down with a diluted Nolvasan solution to remove urine, fecal boli, and other olfactory cues. All animals were tested between 0830 – 1430.

Statistical Analyses

The data were analyzed separately for each of the nine measures of motor activity using a univariate repeated measures ANOVA. The between subjects variable was perchlorate dose, with 5 levels. The three within subjects variables were sex (2 levels), age (3 levels), and time block (9 levels). Due to violation of the sphericity assumption, the more conservative Greenhouse-Geisser test was employed. The fiducial limit was set at $p < 0.05$.

Results and Discussion

Due to attrition, statistical analyses were completed for 84 litters of the original 110 dams: 15 control litters, 18 at 0.1 mg/kg/day, 19 at 1.0 mg/kg/day, 17 at 3.0 mg/kg/day, and 15 at 10.0 mg/kg/day. The average dose consumed for each dose group throughout the study is provided in Table 1, indicating acceptable approximations of the target doses.

No statistically significant differences were found for the main effect of drug dose for any of the 9 measures of motor activity, and there were no reliable interactions related to drug dose. This suggests minimal effect of ammonium perchlorate on the measures of rat pup general locomotor activity studied here. However, a general pattern in the results shows that, in several instances, there was a notable divergence in activity between the control group versus dosed groups, and this difference emerged late in the 90-minute testing sessions (see Figures 1 - 27). An avenue for future research is to specifically evaluate the rate of habituation to a testing situation such as the open field. Patterns in the present data, and in a previous study (York, 1998) suggest that exposed pups may have a slightly slower rate of habituation, and thus maintain a higher level of activity as compared to untreated pups.

As expected, there was a main effect for age in all 9 measures of motor activity. In most cases, there was an increase in locomotion from PND14 to PND18, and a slight reduction from PND18 to PND22 (Table 2). The only measures that deviated from this pattern were rears and movement in the vertical plane where a consistent increase was observed with increasing age. A

reliable main effect for time block was also found for all 9 dependent variables due to an overall decrease in behavior from start to finish of each test session (Table 3). The time-dependent reduction in motor activity was less evident on PND14 and PND18, than on PND22, as indicated in the significant 2-way, age x time block, interaction (Table 4). The interaction was reliable for all motor activity measures, however, the measures of rears and vertical plane movements were not consistent with this general pattern. Rather, for these two measures, decreases from the start to finish of the test sessions were found on PND14 and PND22, but on PND18 the number of rears was the same at the end as at the beginning of the 90 minutes, and vertical plane movements increased from beginning to the end of the session.

The three-way interaction of sex x age x time block was significant for some measures, specifically, time ambulatory, stereotypic bursts, stereotypic time, horizontal movements, and time resting. The pattern of behaviors in these separate groups are found in Figures 4-6, 7-9, 13-15, 16-18, and 25-27, respectively. Overall, the primary difference between the females and males is found on PND14 during the time blocks near the midpoint of the 90-minute test session. For the measures of time ambulatory, stereotypic bursts, and horizontal movements, the females demonstrated a slight decrease in the behavior while the males demonstrated a slight increase. The inverse pattern was found for time resting.

For the measure of vertical plane movements, there was a significant sex x time block interaction. The effect was due to a greater decrease in these movements during the earlier half of the test session in females as compared to the males. However, the effect was not reliable for any of the other dependent measures.

Conclusion

In conclusion, the results suggest a pregnant dam's exposure to ammonium perchlorate does not reliably affect the development of gross motor movements in her pups. The integrity of the neural system for motor behavior was demonstrated at three post-weaning ages, where no differences were found between any of the dose groups for a variety of motor activity variables. Although previous research suggests the exposure may cause abnormalities in cerebellar

development, which could be manifested in changes of locomotor behavior, the current evidence does not support such a prediction. However, a pattern did emerge suggesting there may be subtle changes in habituation to the testing environment related to previous ammonium perchlorate treatment. A similar pattern was reported in results from a closely related investigation (York, 1998). In such cases, a treatment effect would be less due to the integrity of the neural substrates specific for motor activity, and more likely related to a general brain system for behavioral inhibition. An appropriate follow-up to the present research would be to employ neurobehavioral tasks to specifically investigate habituation to novel stimuli or environments. For instance, future studies may use new animals for testing at each of the different ages in order to maximize the novelty of the testing situation. Such methodology may serve to increase the sensitivity of the open field locomotor activity test to changes in the animals' patterns of habituation, as well as indicate the integrity of the motor system.

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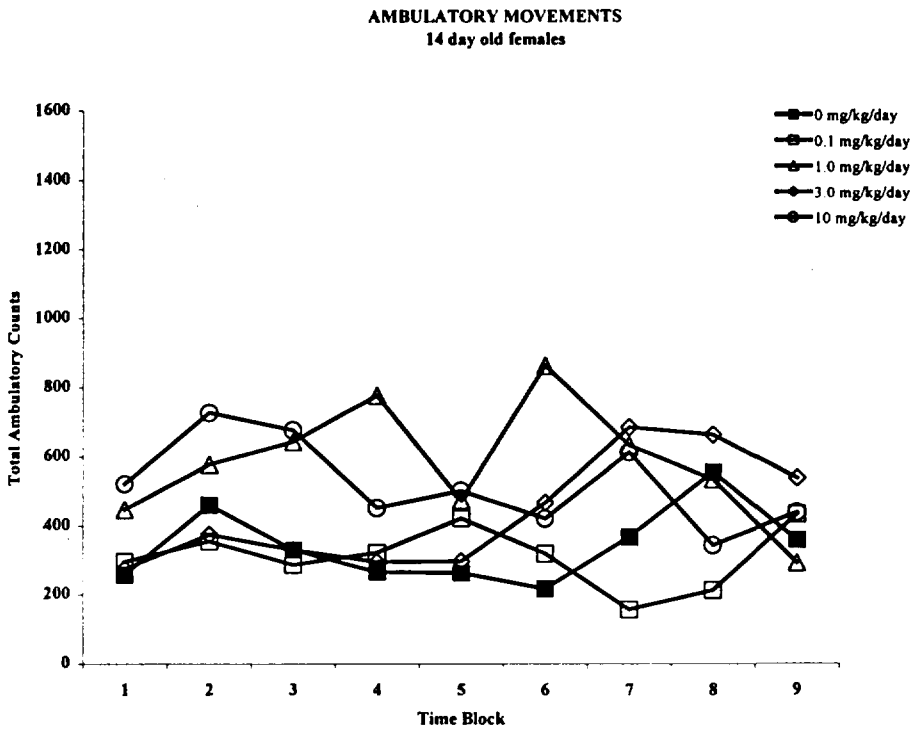
Figure 1.


AMBULATORY MOVEMENTS
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0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Total Ambulatory Counts
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1000
800
600
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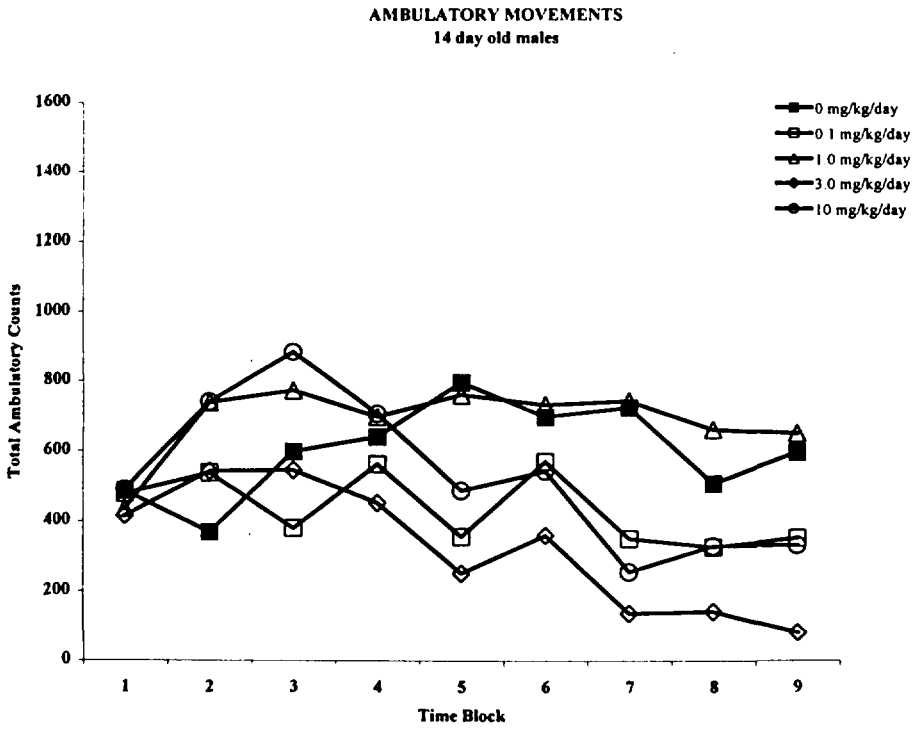
1
2
3
4
5
6
7
8
9
Time Block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14 day old males
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3.0 mg/kg/day
10 mg/kg/day
Total Ambulatory Counts
1600
1400
1200
1000
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600
400
200
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3
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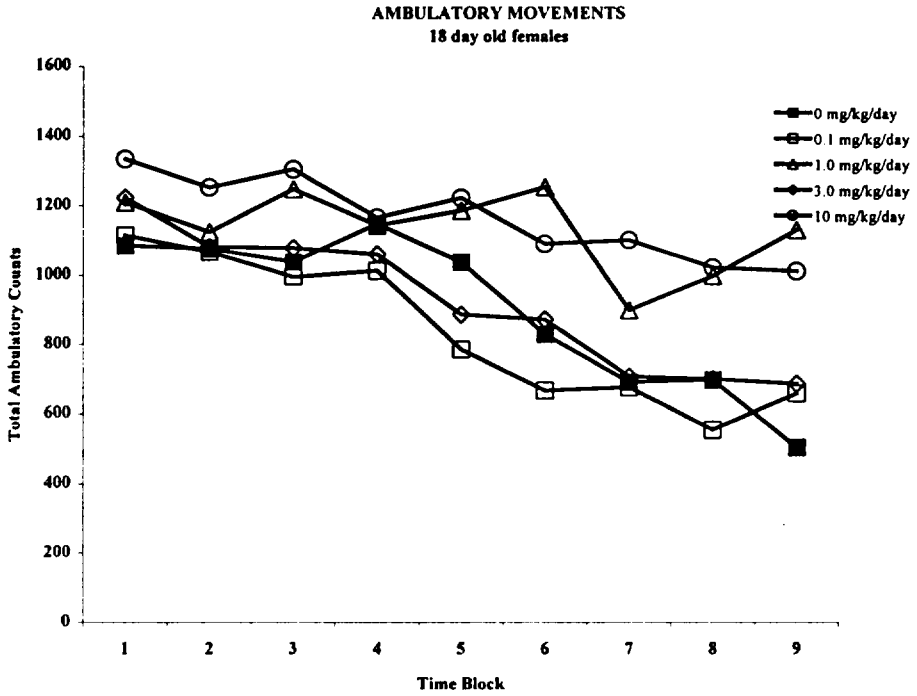
5
6
7
8
9
Time Block

Figure 2.


AMBULATORY MOVEMENTS
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1200
1000
800
600
400
200
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1
2
3
4
5
6
7
8
9
Time Block
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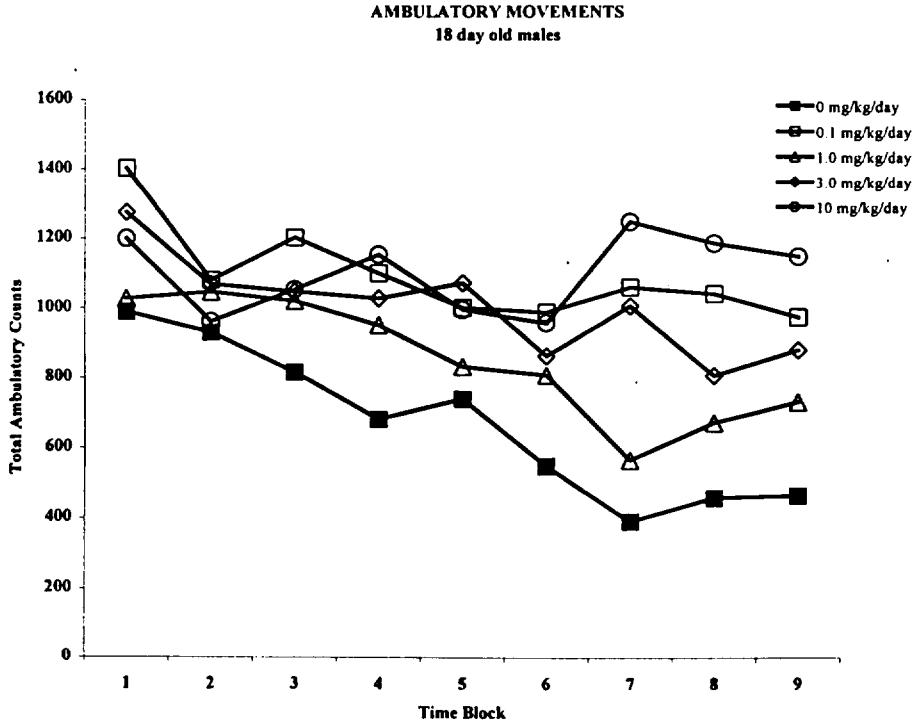
3.0 mg/kg/day
10 mg/kg/day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1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block
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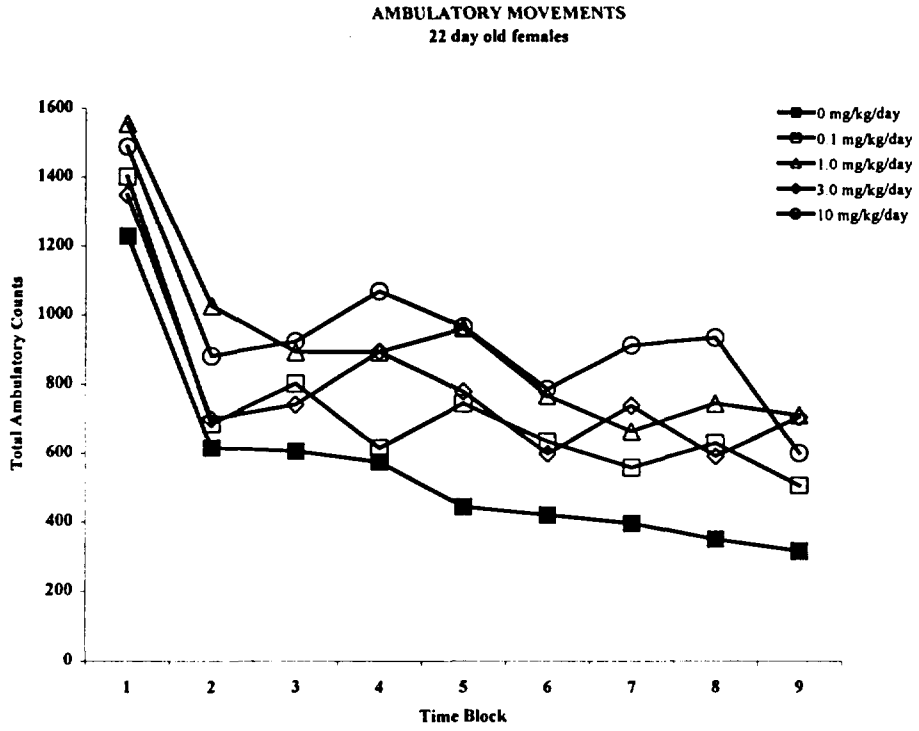
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 3.


AMBULATORY MOVEMENTS
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Total Ambulatory Counts
1600
1400
1200
1000
800
600
400
200
0
1
2
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Time Block

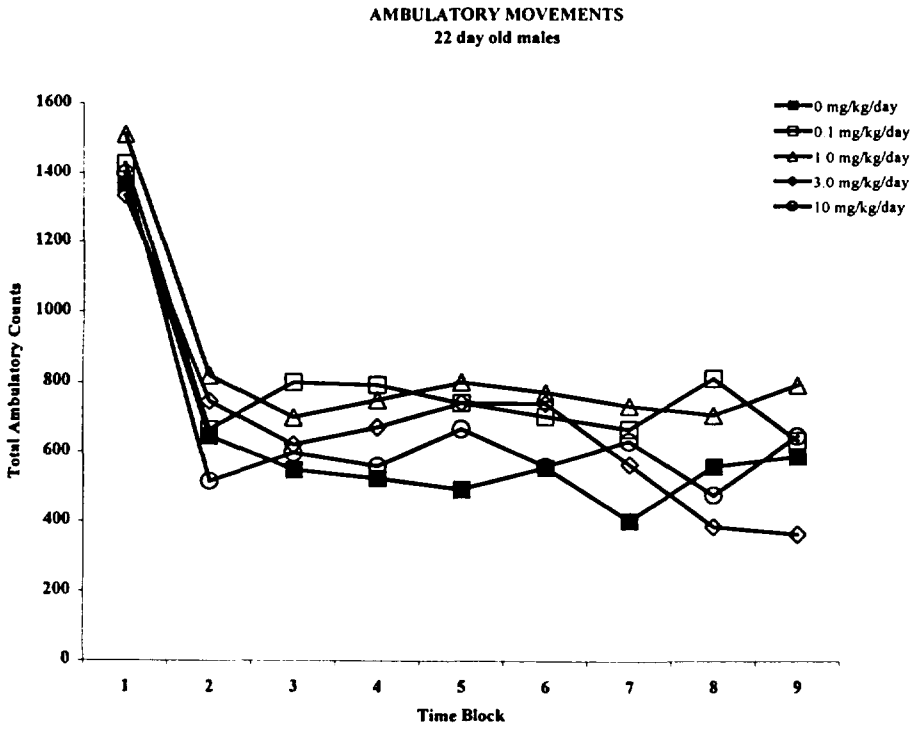
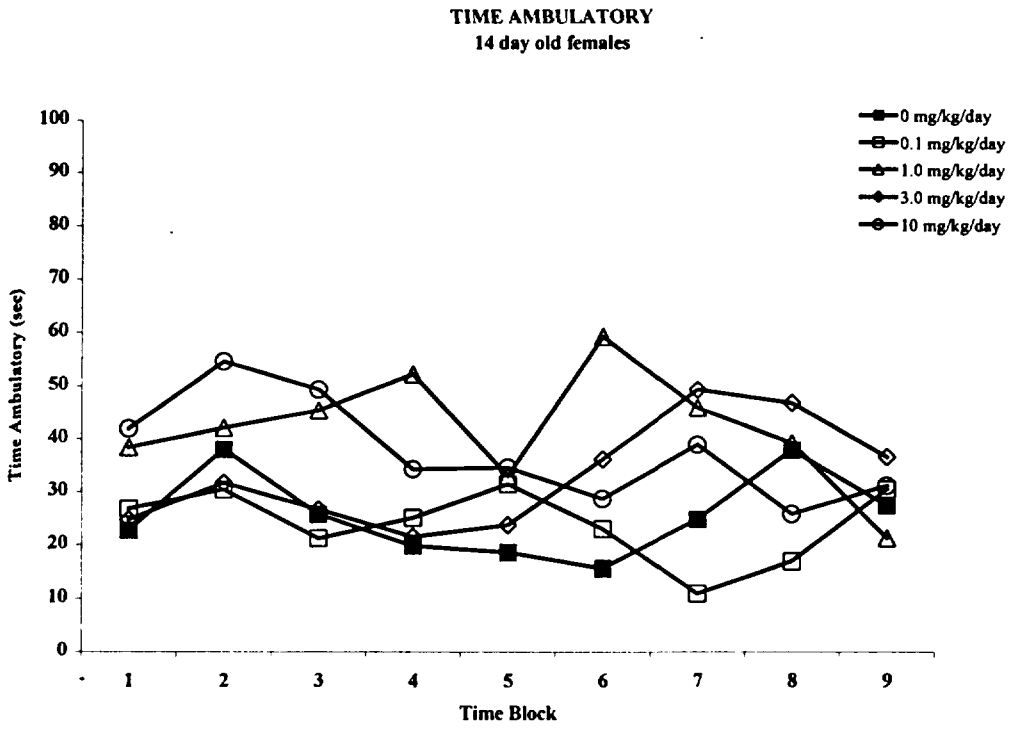

AMBULATORY MOVEMENTS
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Total Ambulatory Counts
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 4.


TIME AMBULATORY
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block

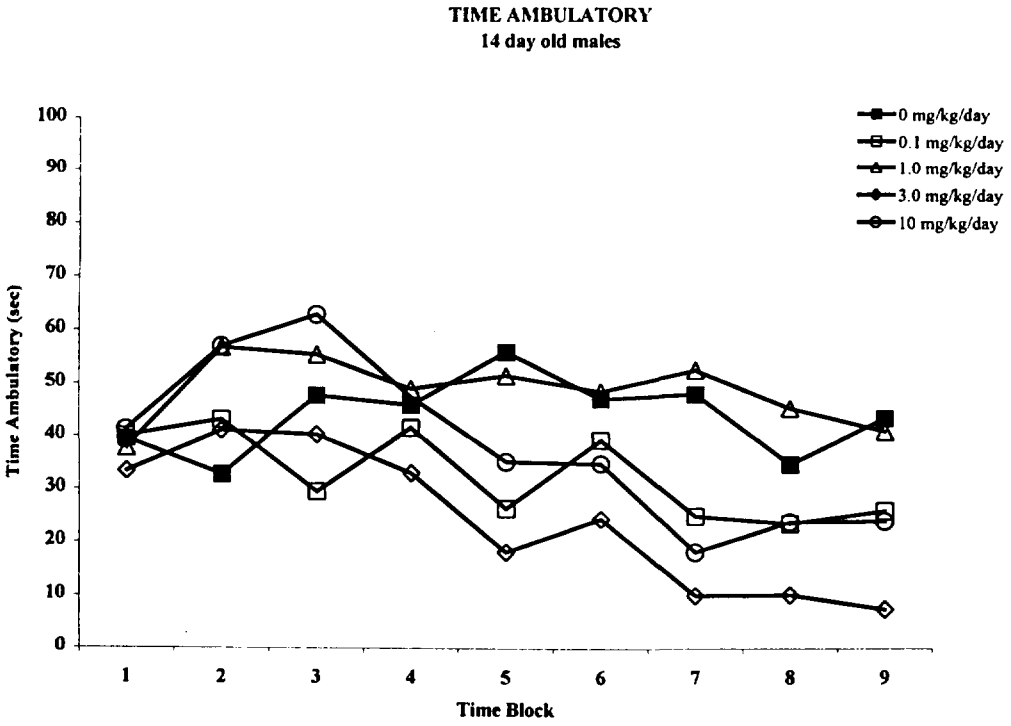
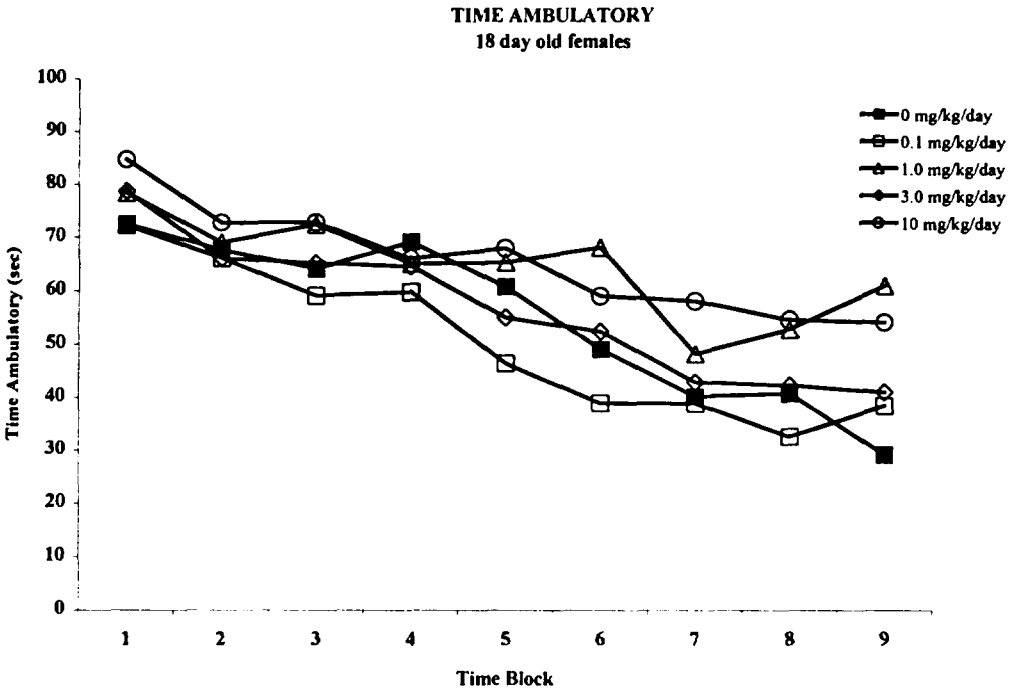

TIME AMBULATORY
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 5.


TIME AMBULATORY
18 day old females
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

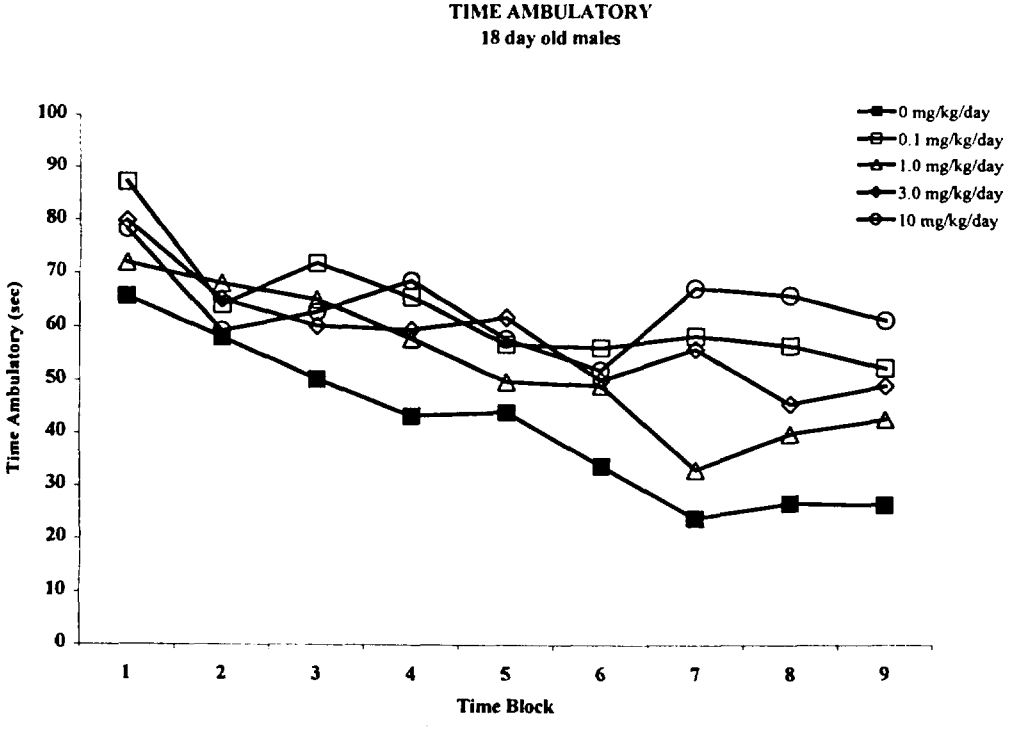
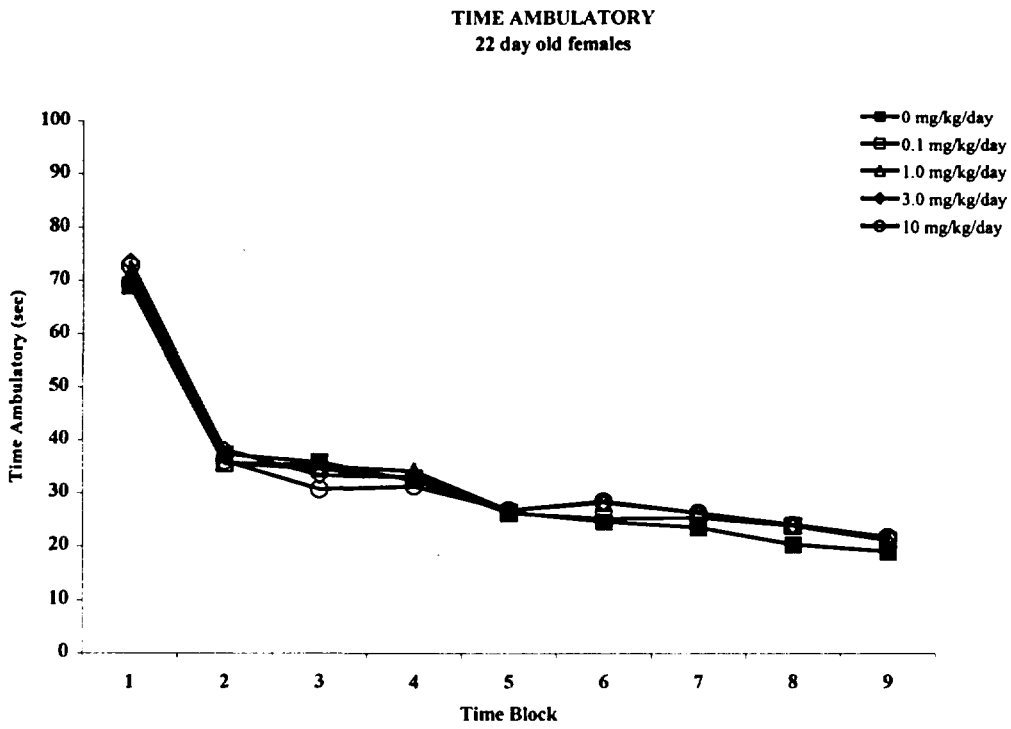

TIME AMBULATORY
18 day old males
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 6.


TIME AMBULATORY
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block

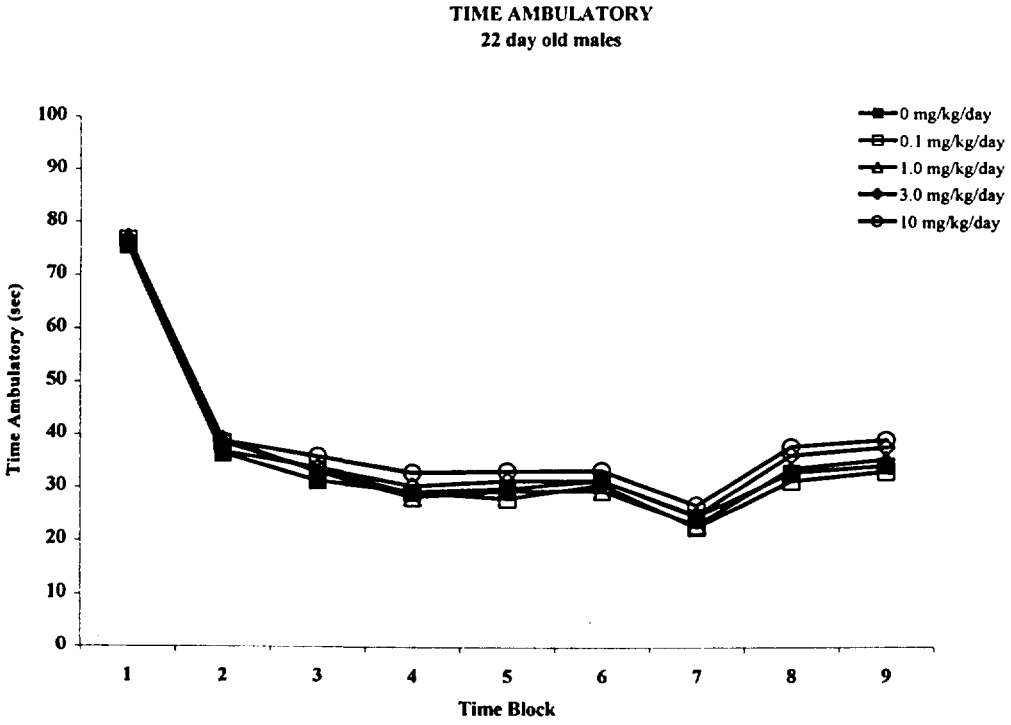
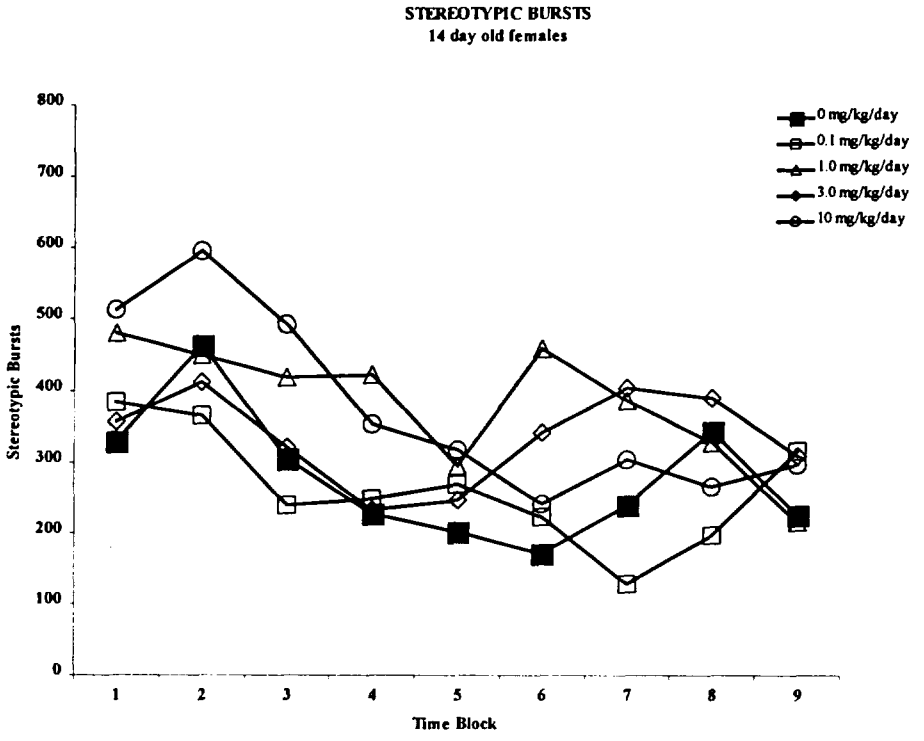

TIME AMBULATORY
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Ambulatory (sec)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 7.


STEREOTYPIC BURSTS
14 day old females
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

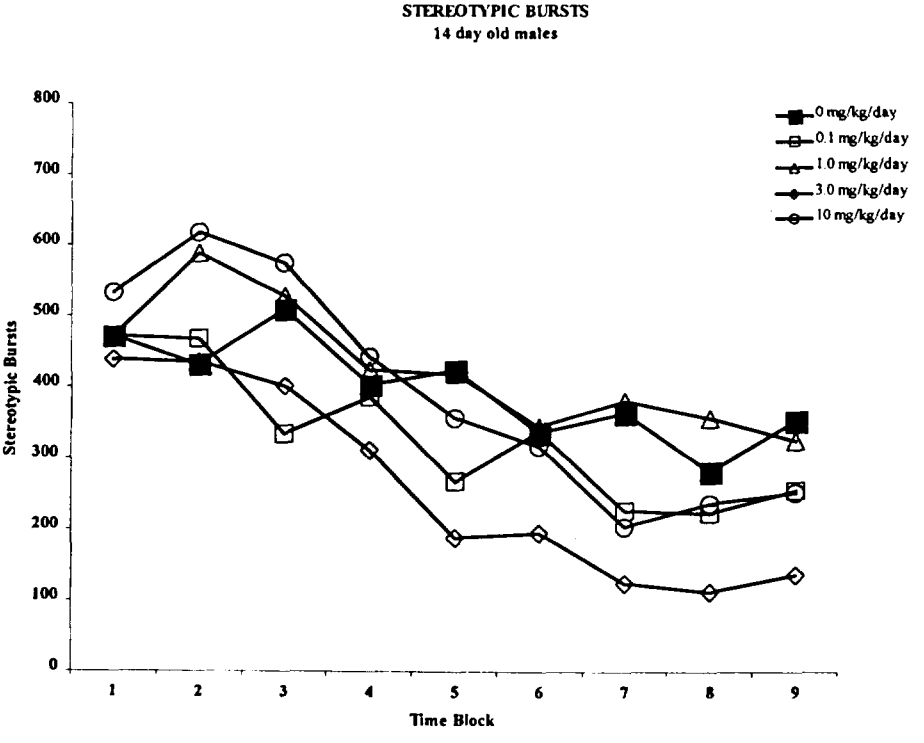
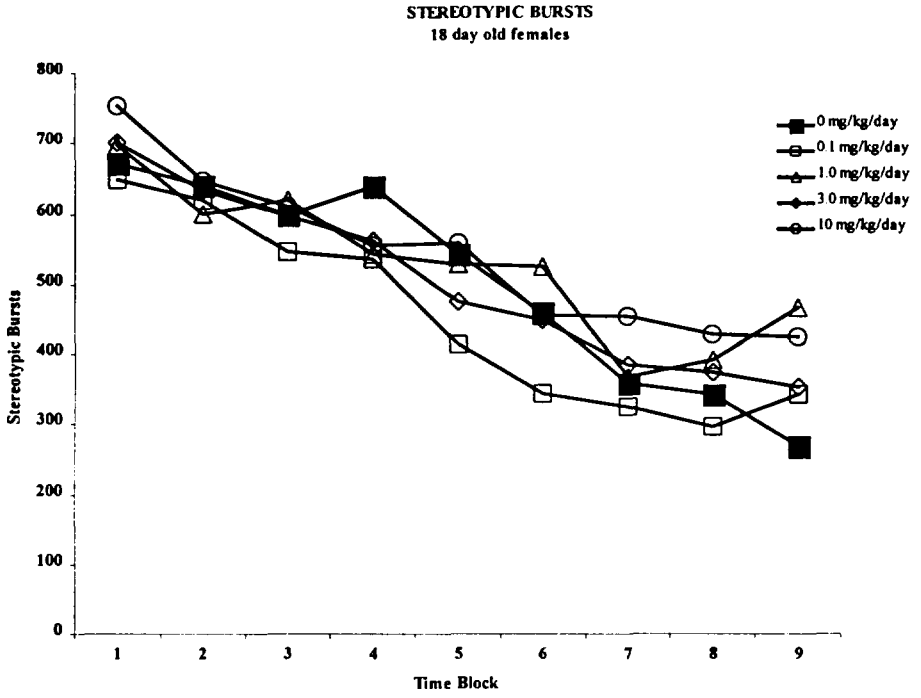

STEREOTYPIC BURSTS
14 day old males
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 8.


STEREOTYPIC BURSTS
18 day old females
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

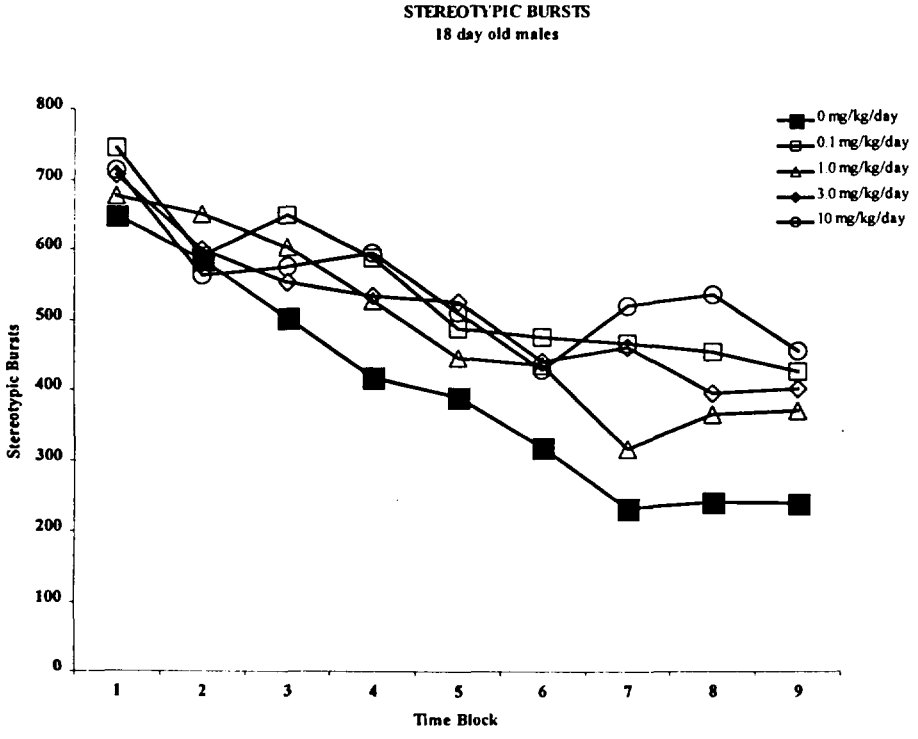
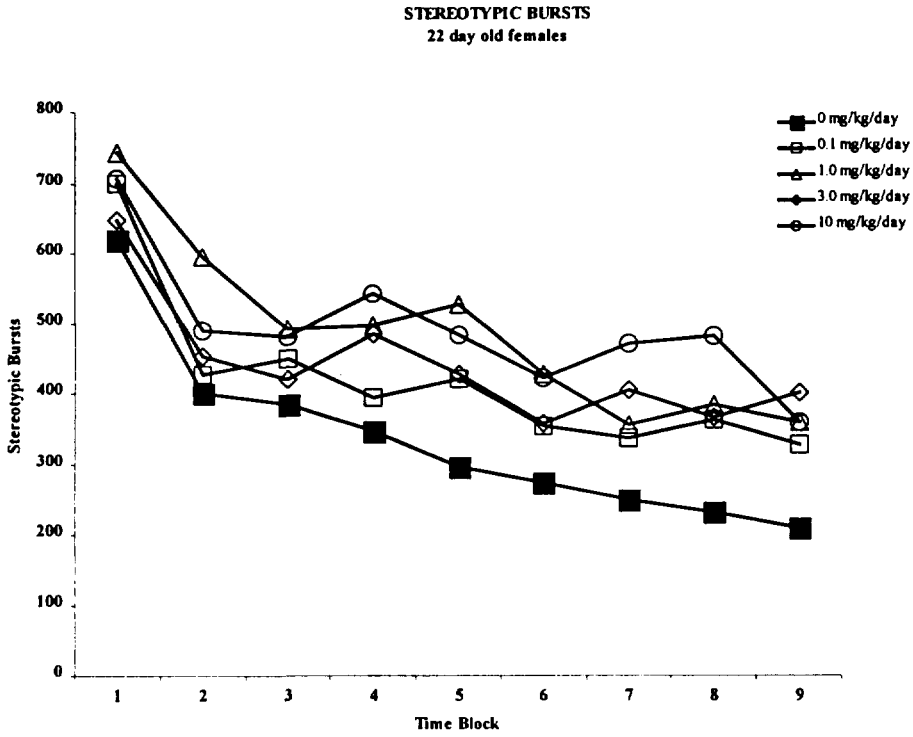

STEREOTYPIC BURSTS
18 day old males
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 9.


STEREOTYPIC BURSTS
22 day old females
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

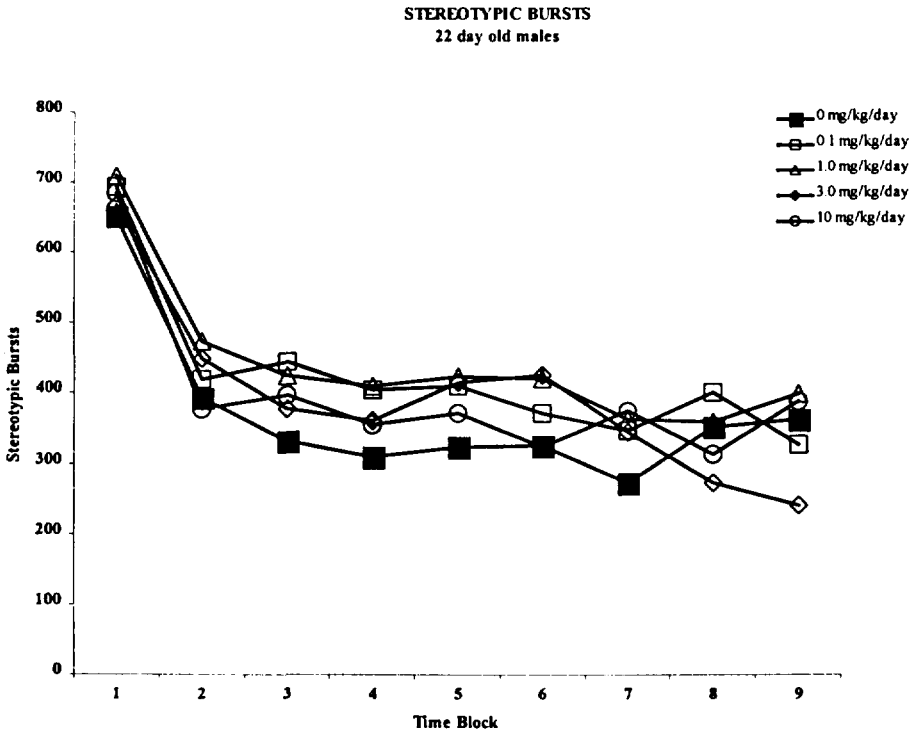
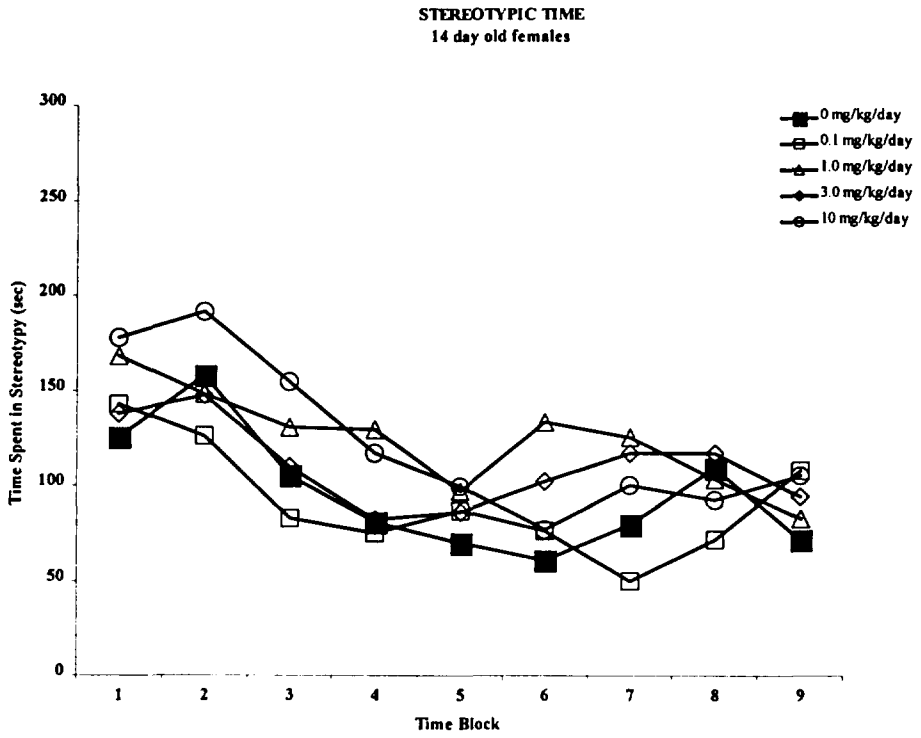

STEREOTYPIC BURSTS
22 day old males
Stereotypic Bursts
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 10.


STEREOTYPIC TIME
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

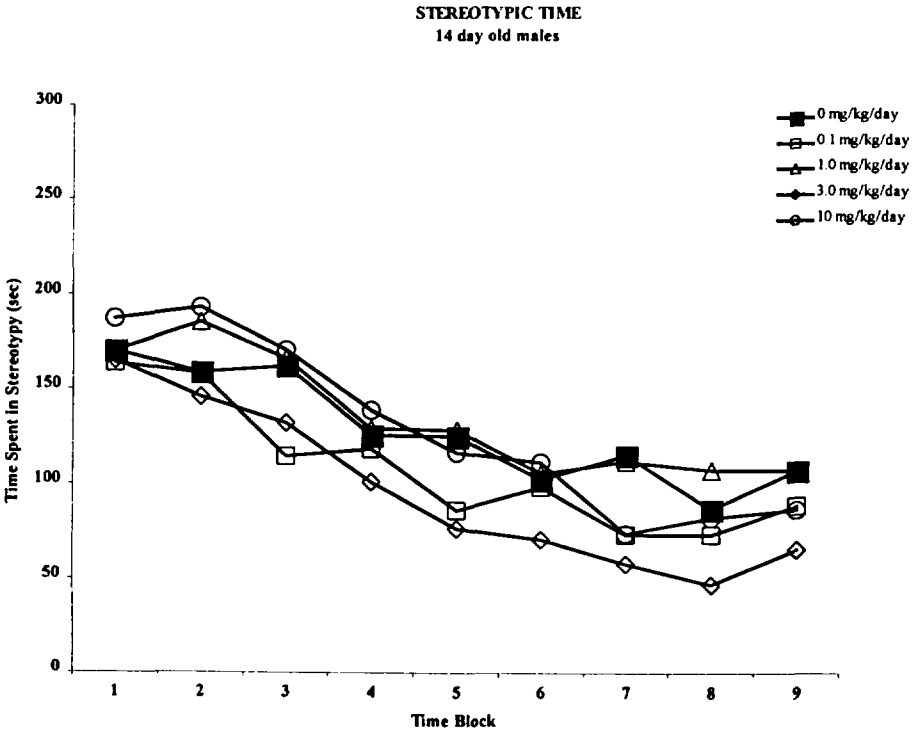
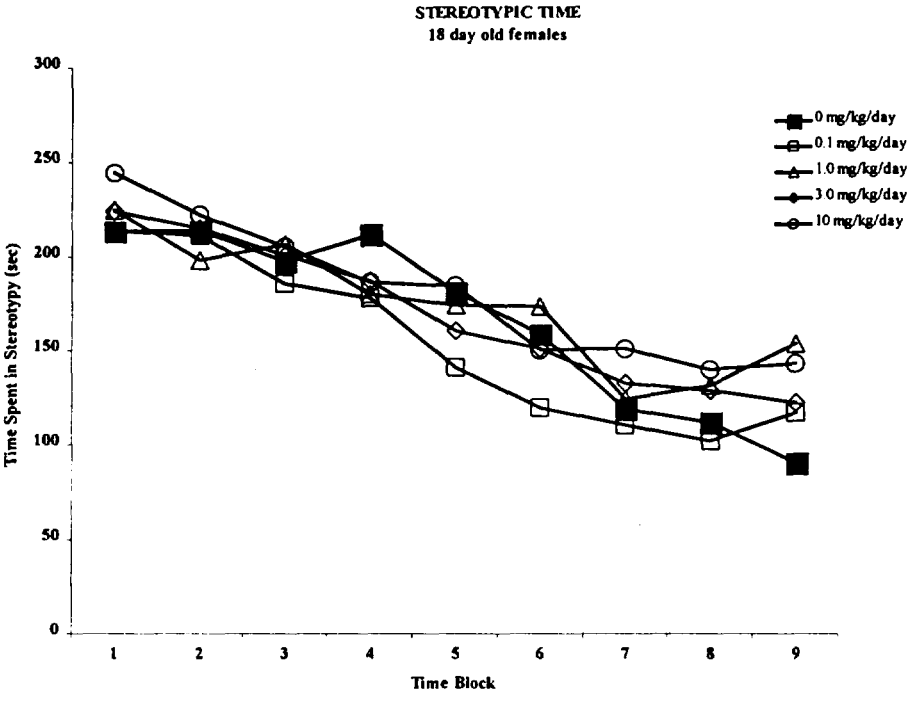

STEREOTYPIC TIME
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 11.


STEREOTYPIC TIME
18 day old females
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

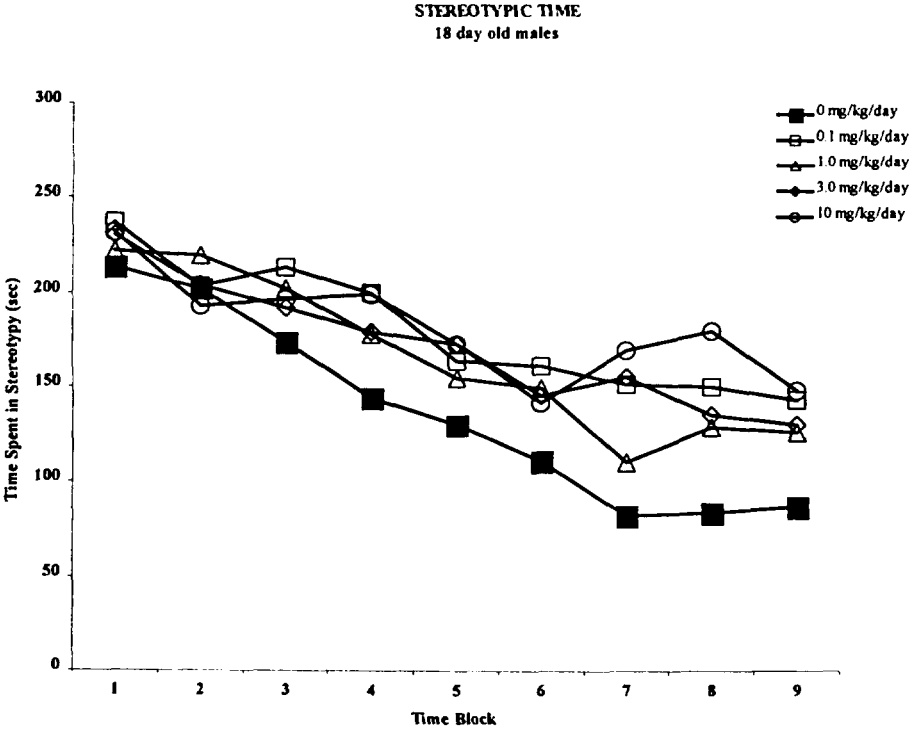
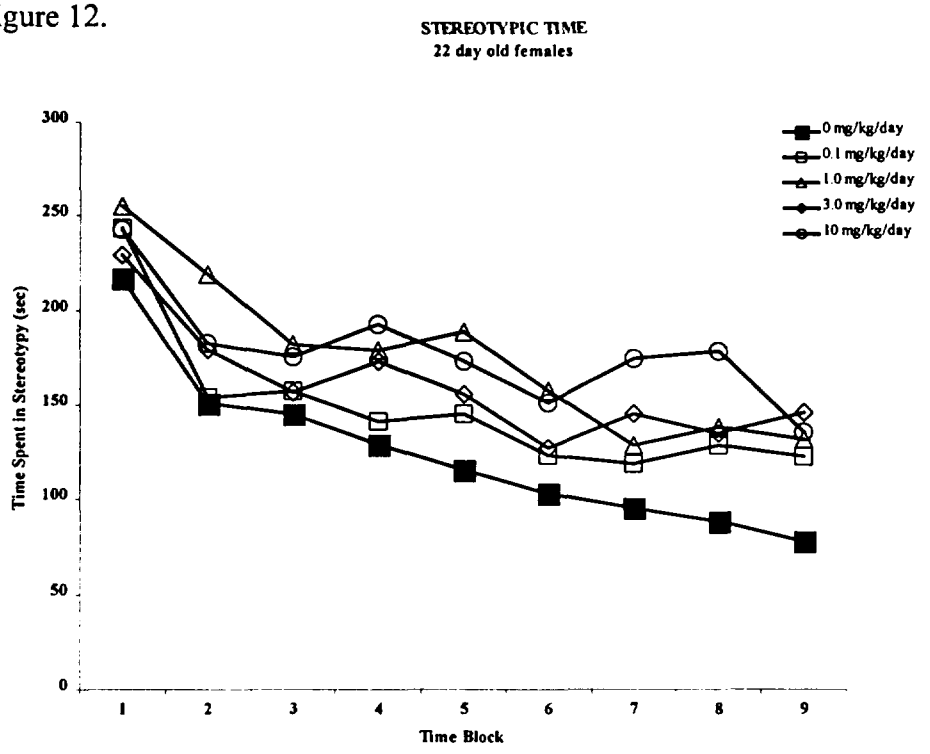

STEREOTYPIC TIME
18 day old males
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 12.


STEREOTYPIC TIME
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

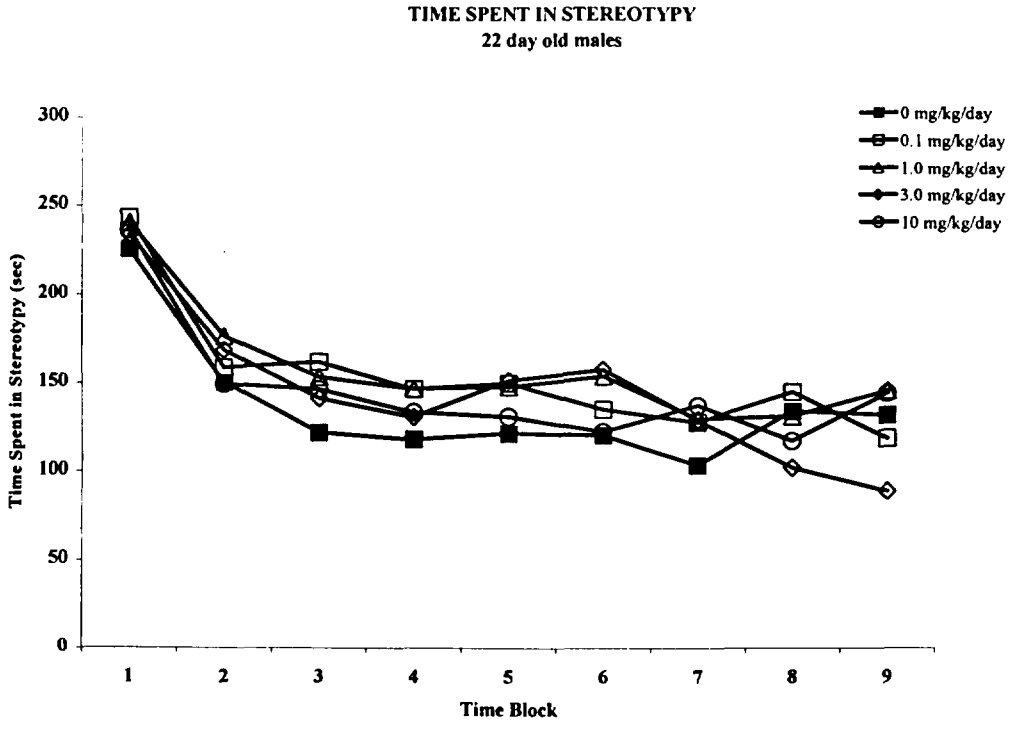
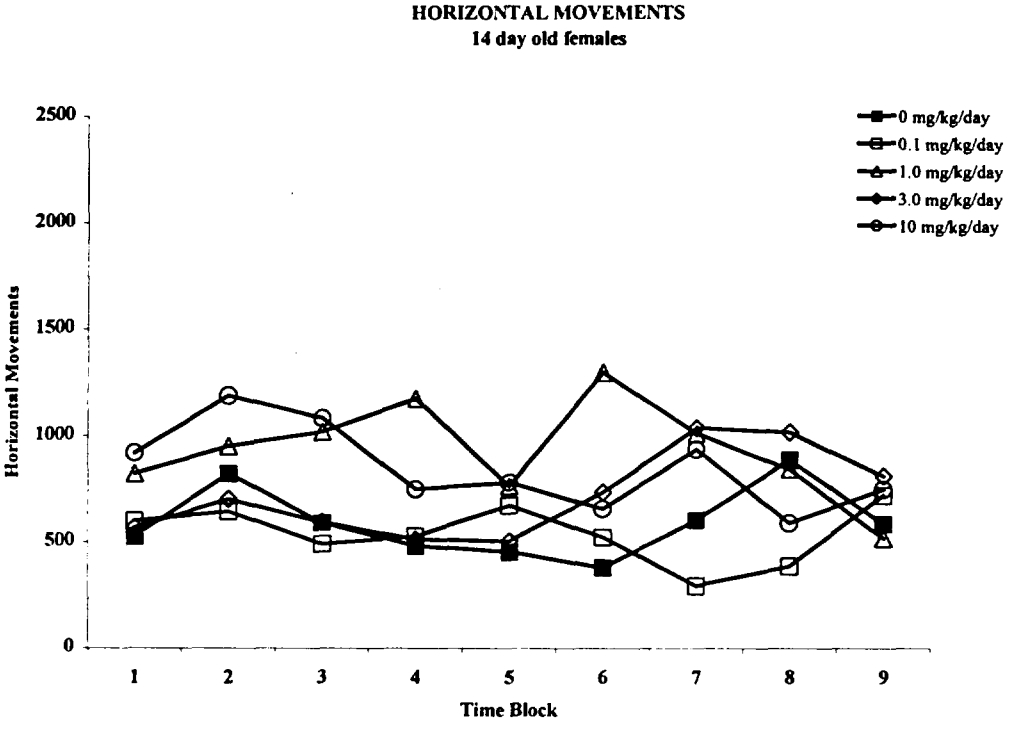

TIME SPENT IN STEREOTYPY
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Spent in Stereotypy (sec)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 13.


HORIZONTAL MOVEMENTS
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block

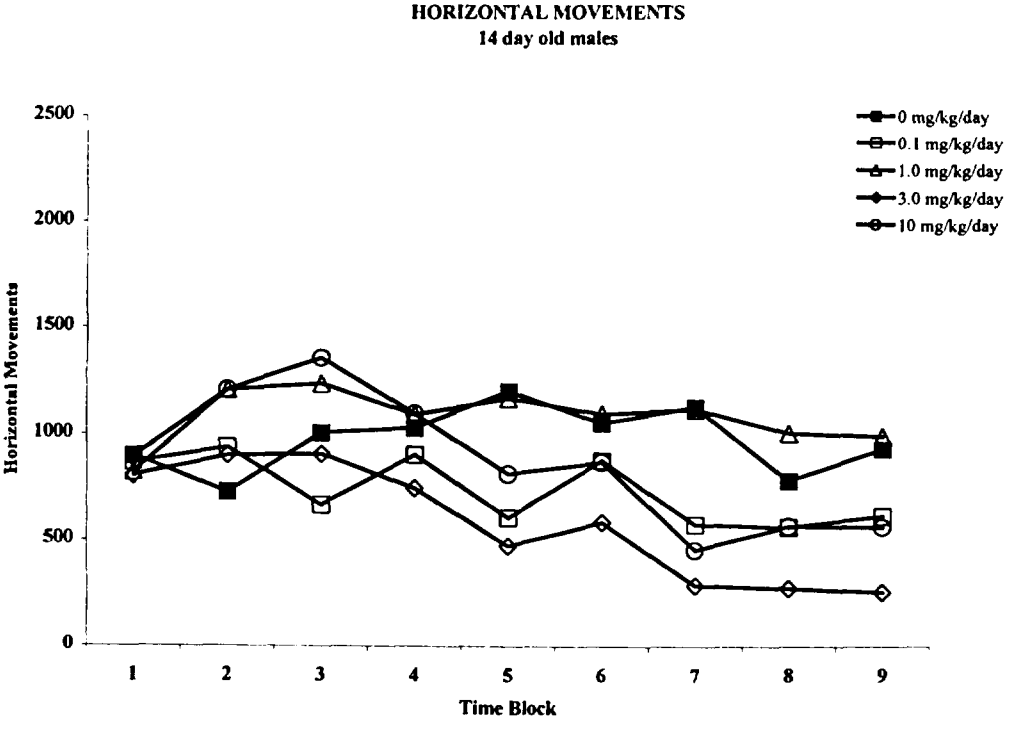
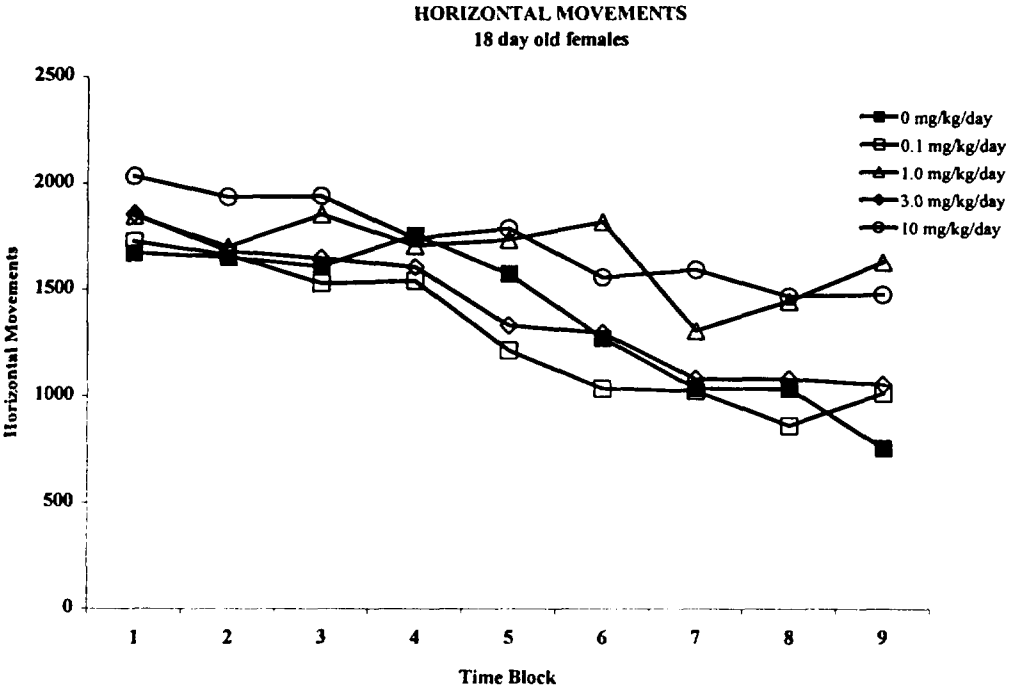

HORIZONTAL MOVEMENTS
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 14.


HORIZONTAL MOVEMENTS
18 day old females
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

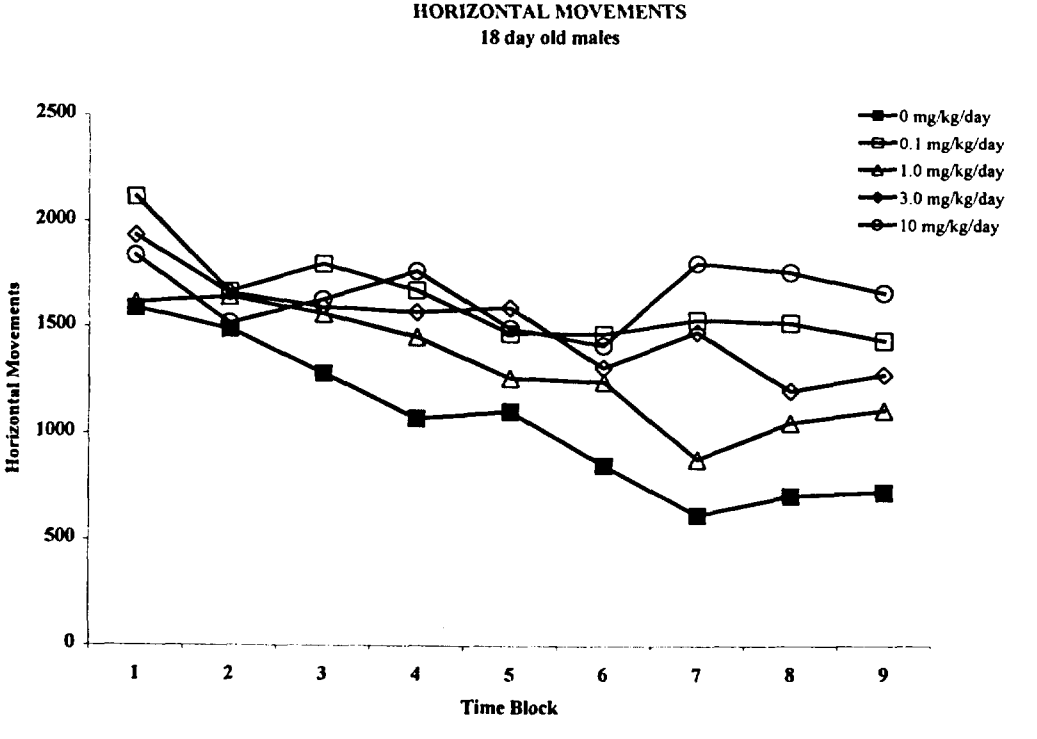
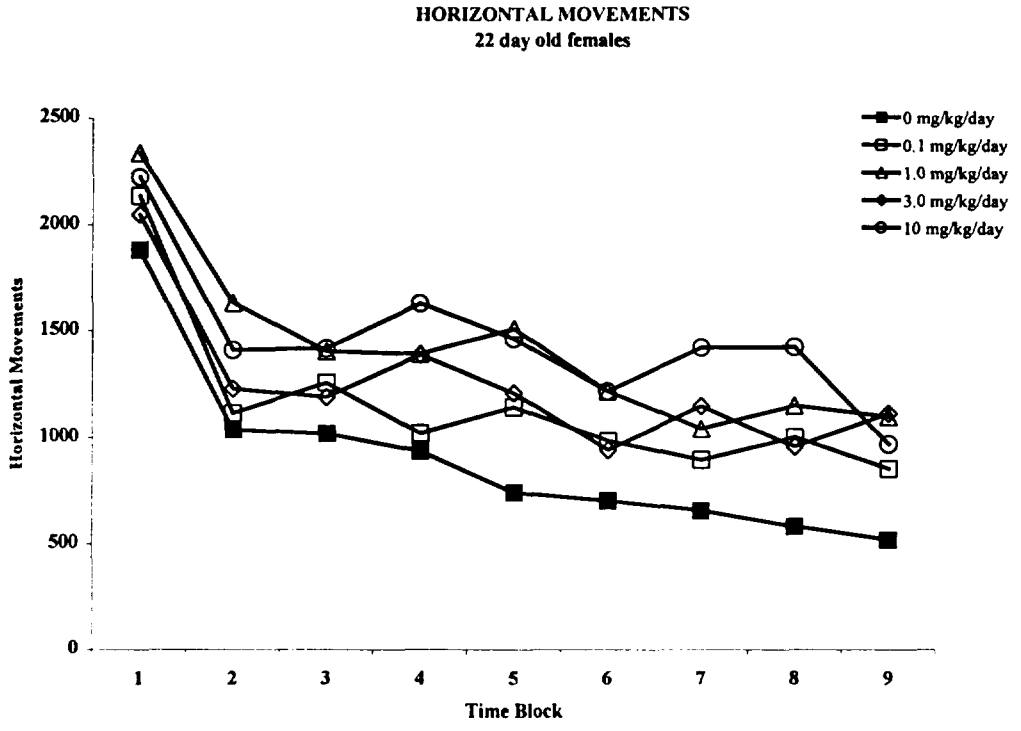

HORIZONTAL MOVEMENTS
18 day old males
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 15.


HORIZONTAL MOVEMENTS
22 day old females
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

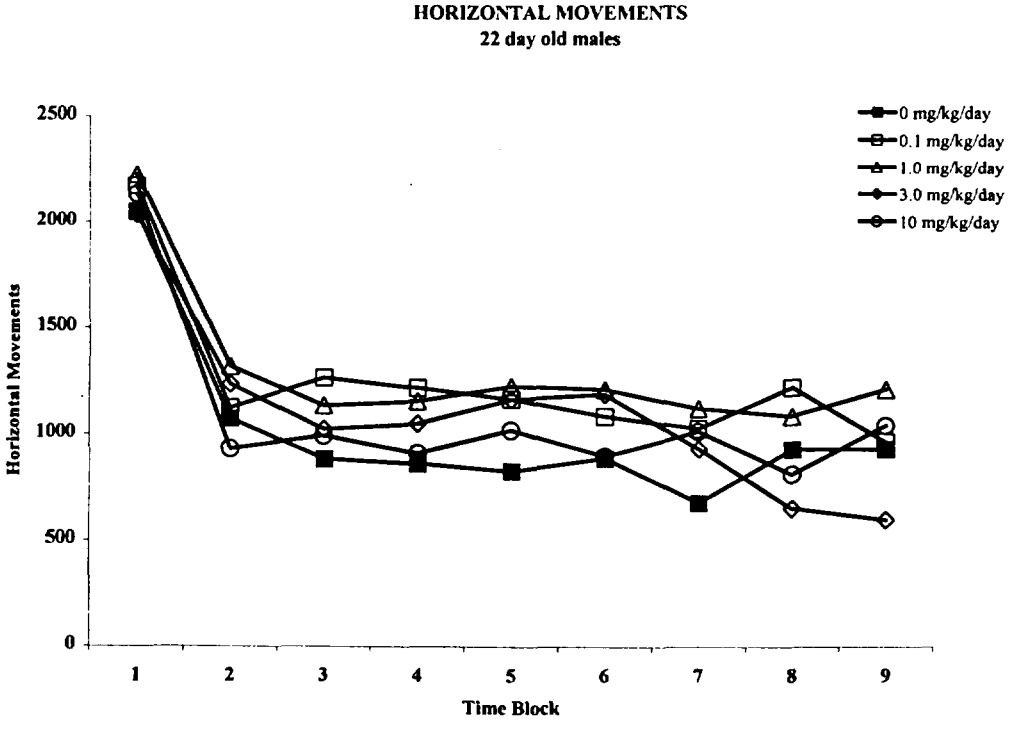
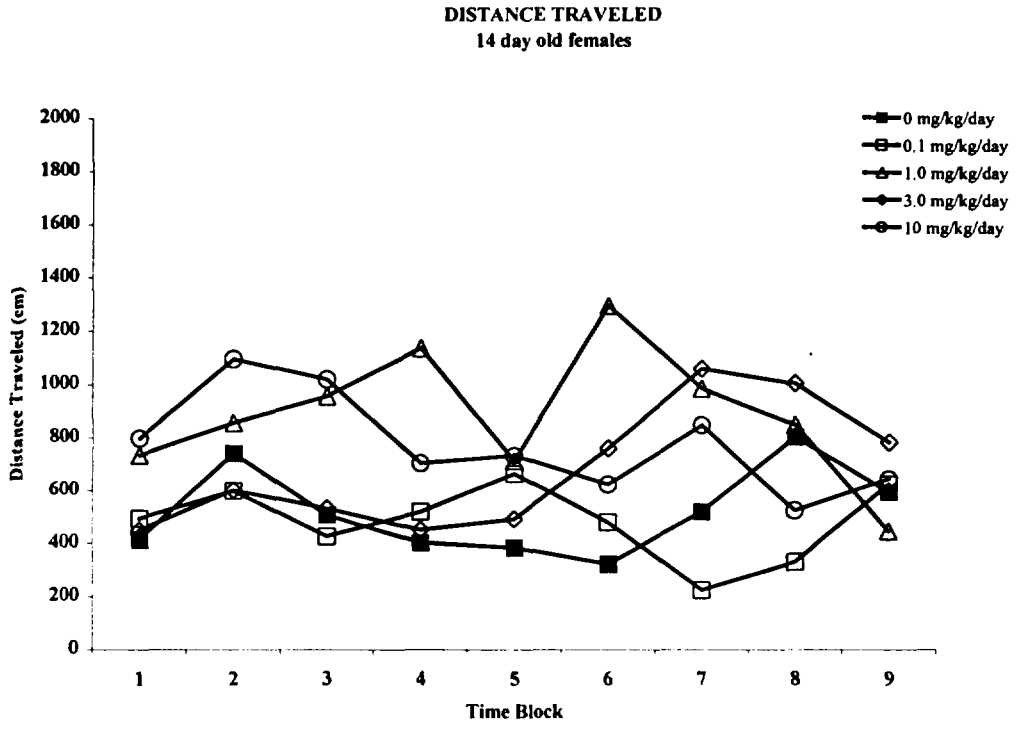

HORIZONTAL MOVEMENTS
22 day old males
Horizontal Movements
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 16.


DISTANCE TRAVELED
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block

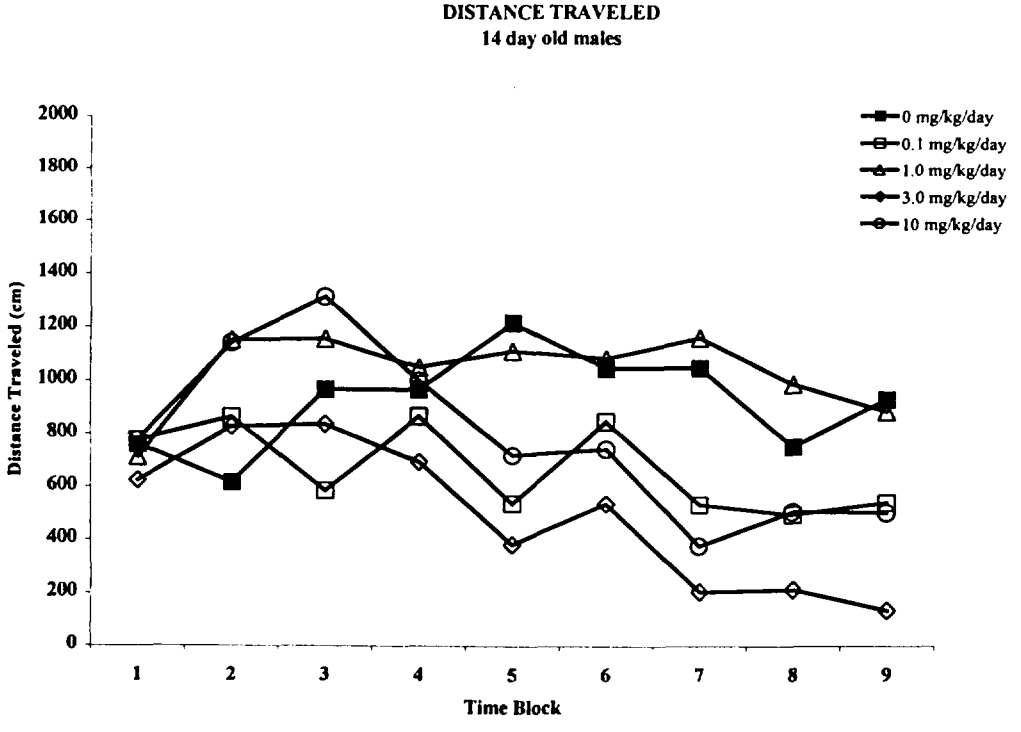
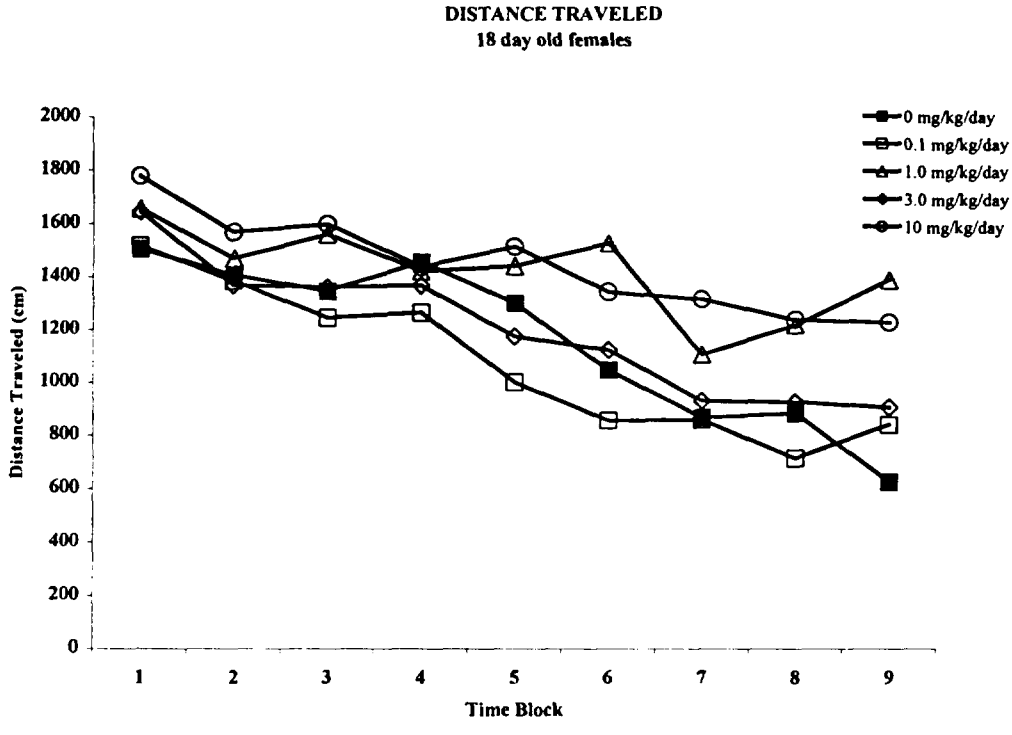

DISTANCE TRAVELED
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 17.


DISTANCE TRAVELED
18 day old females
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

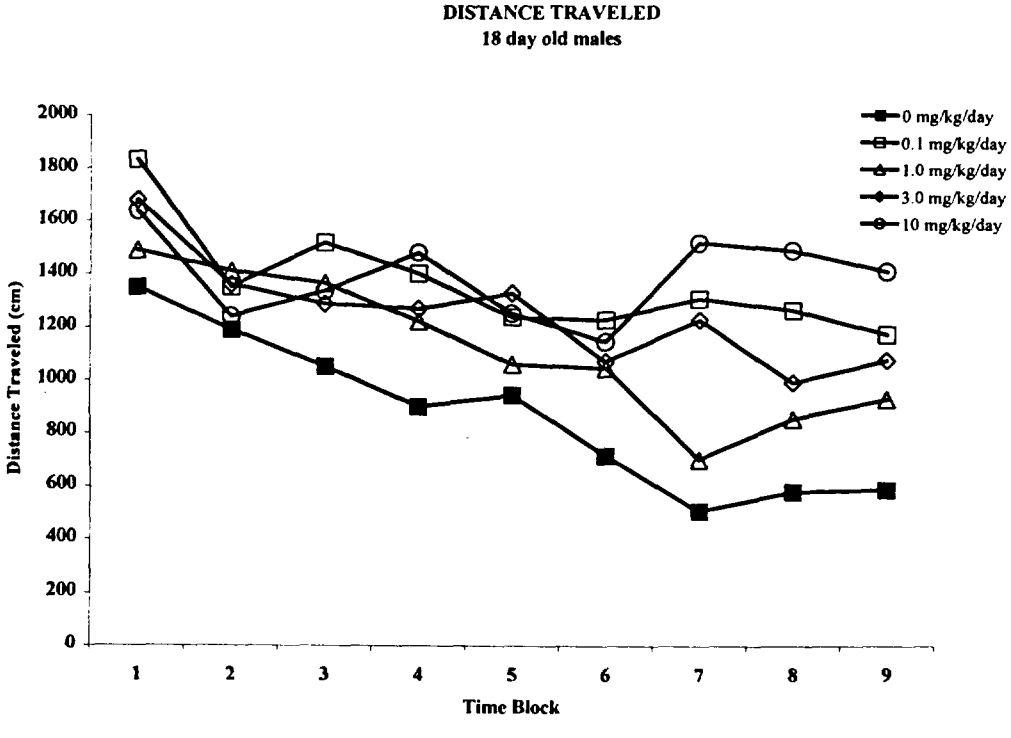
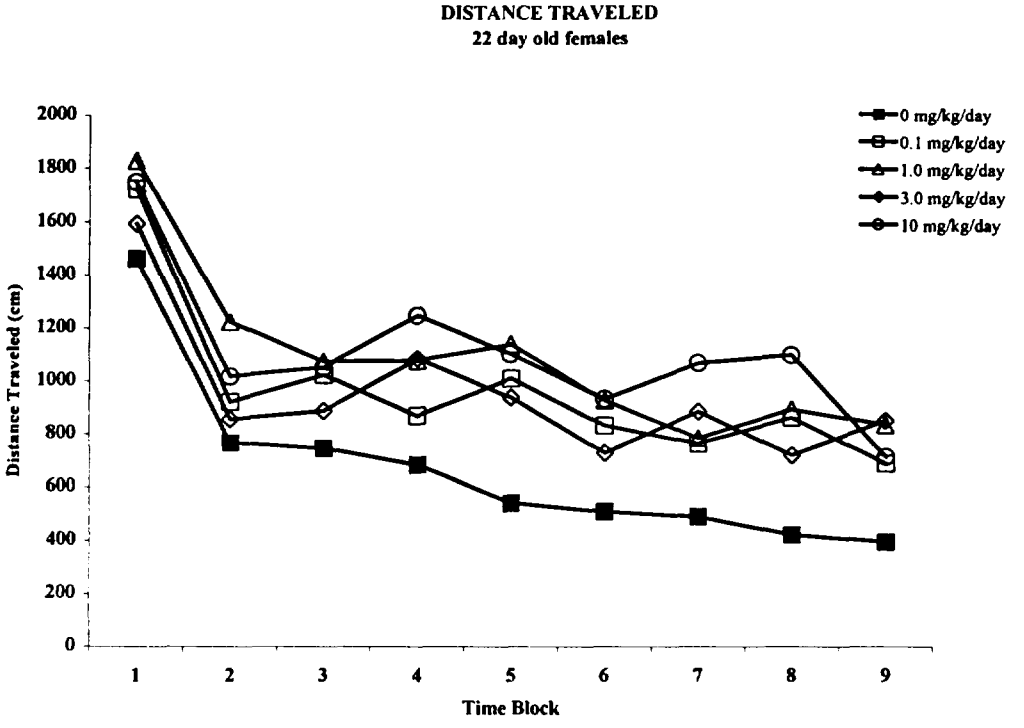

DISTANCE TRAVELED
18 day old males
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 18.


DISTANCE TRAVELED
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block

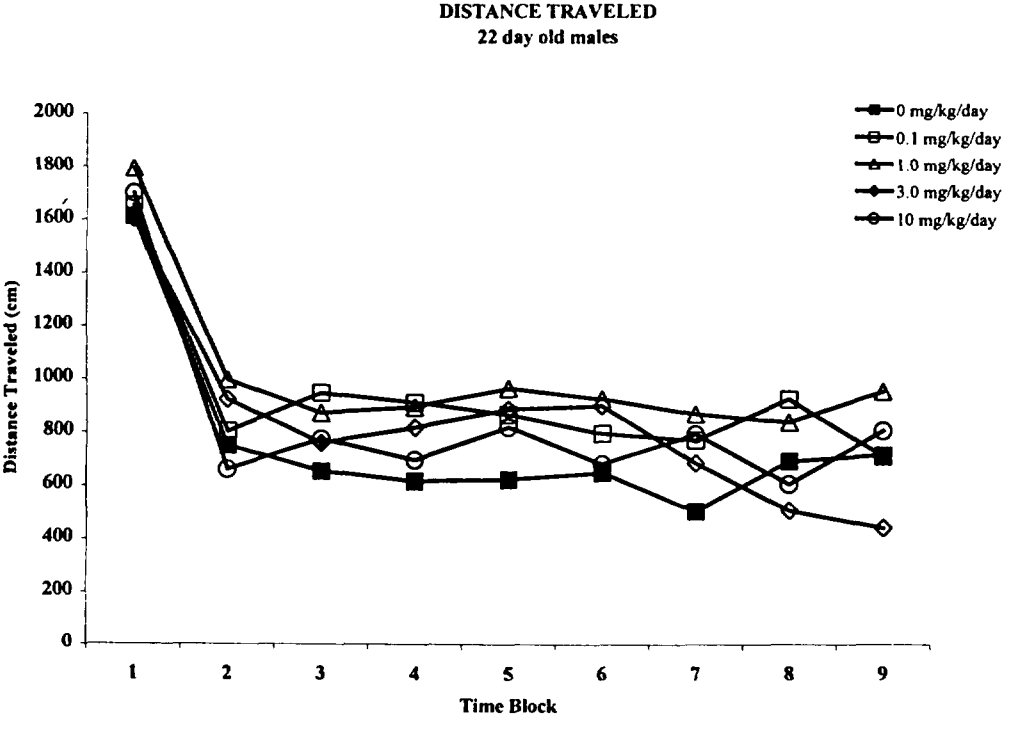
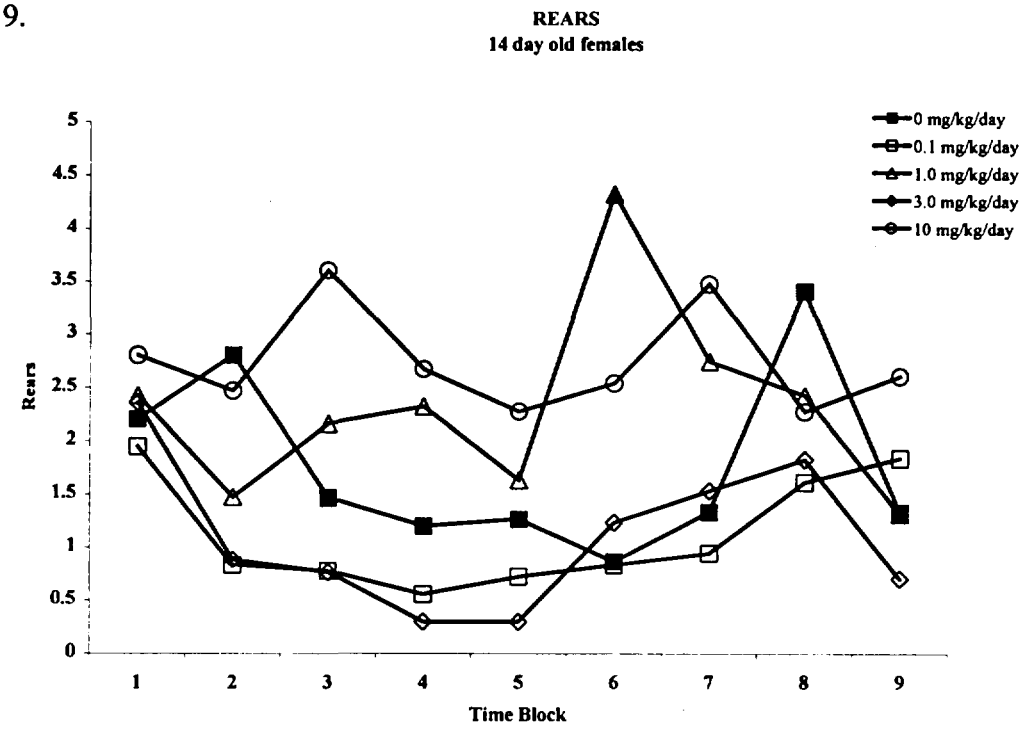

DISTANCE TRAVELED
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Distance Traveled (cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 19.


REARS
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Rears
Time Block

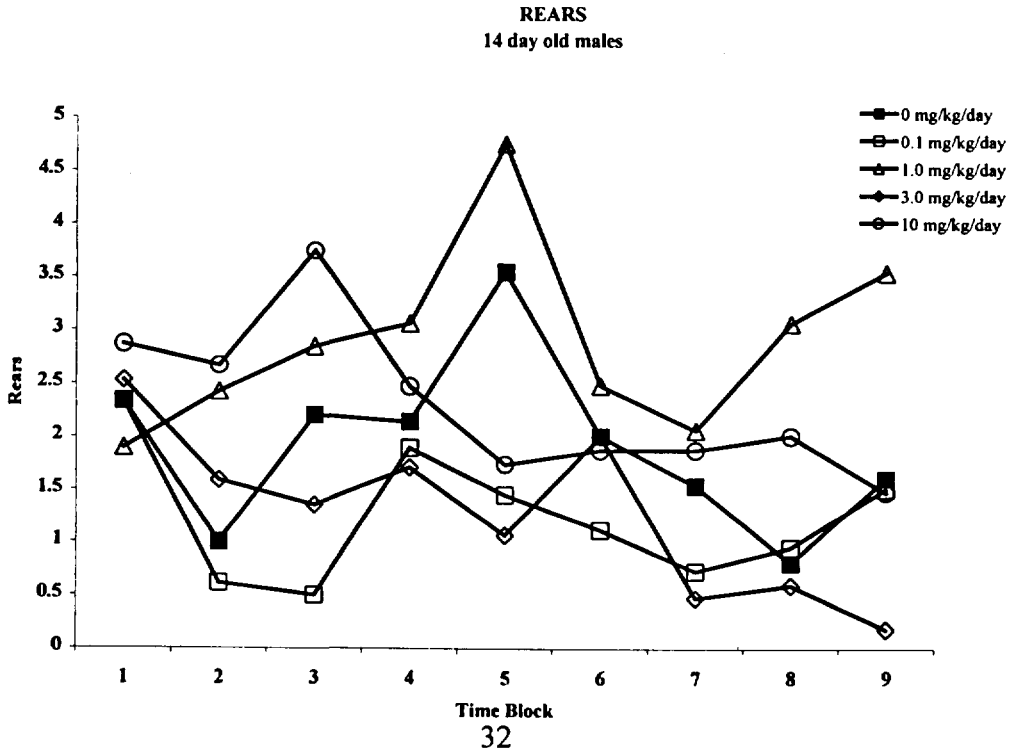
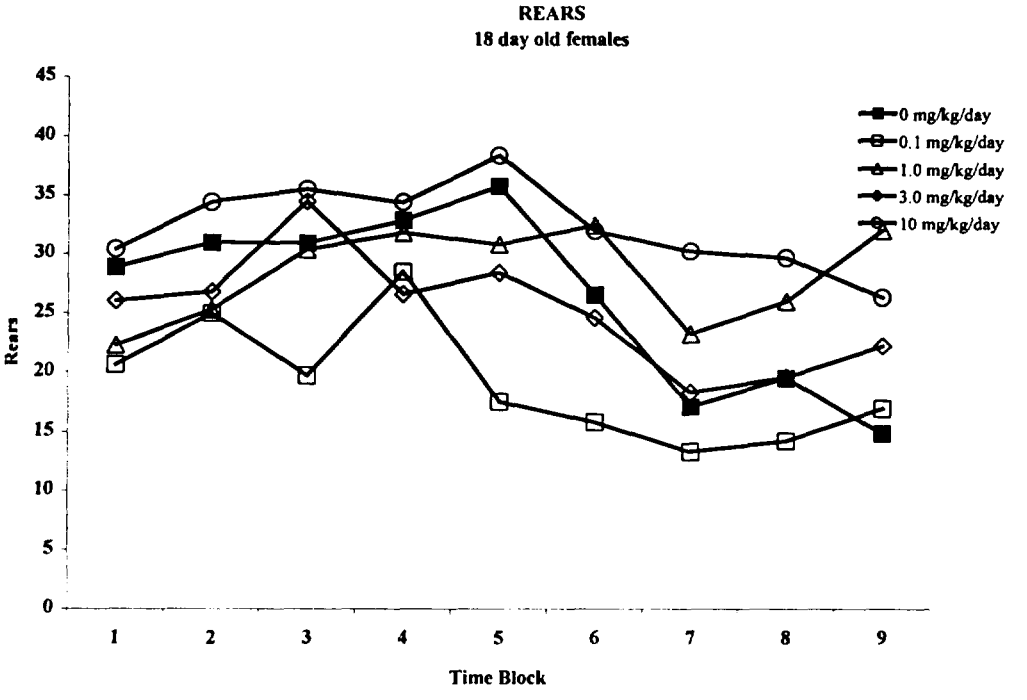

REARS
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Rears
Time Block

Figure 20.


REARS
18 day old females
Rears
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

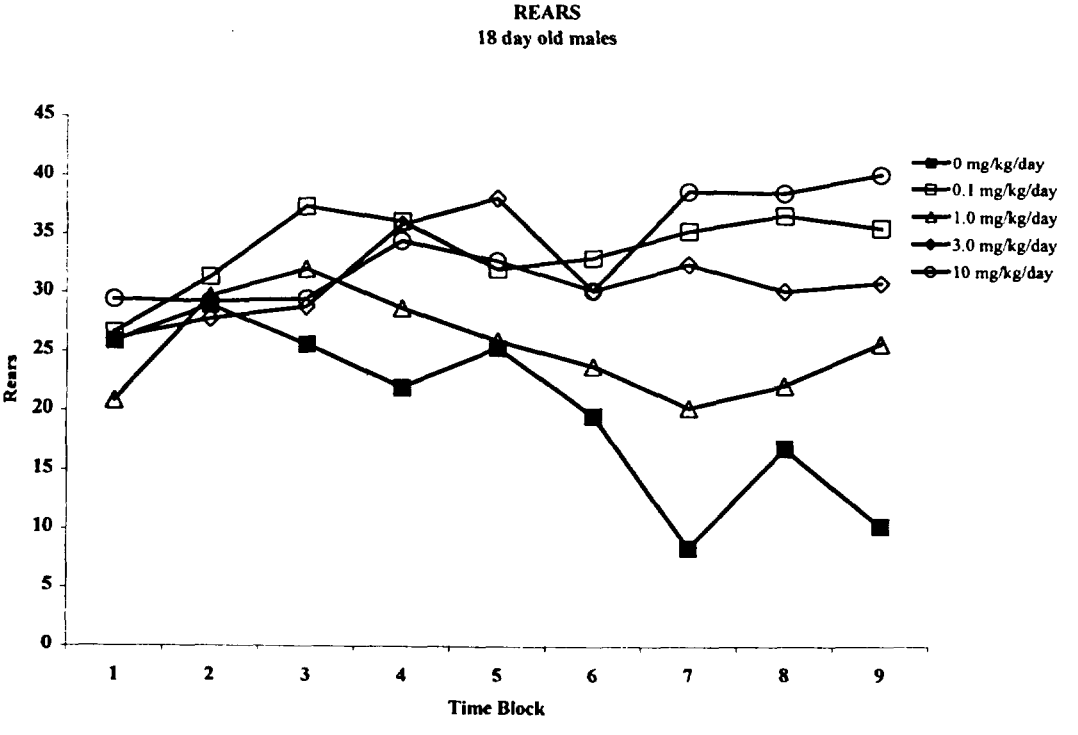
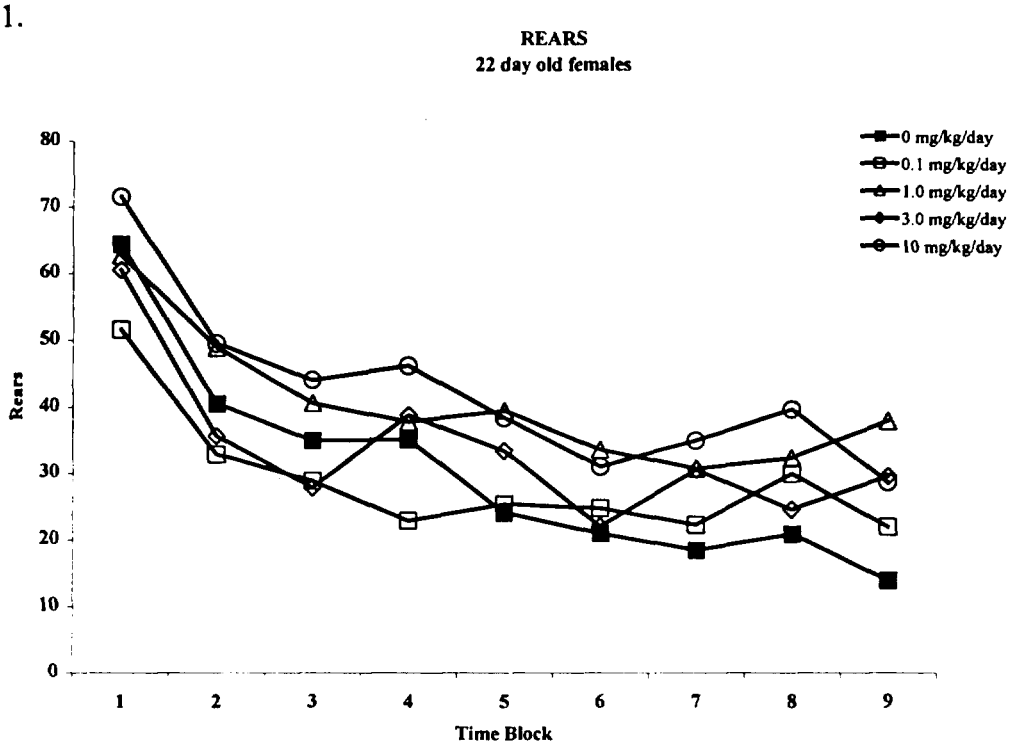

REARS
18 day old males
Rears
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 21.


REARS
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Rears
Time Block
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9

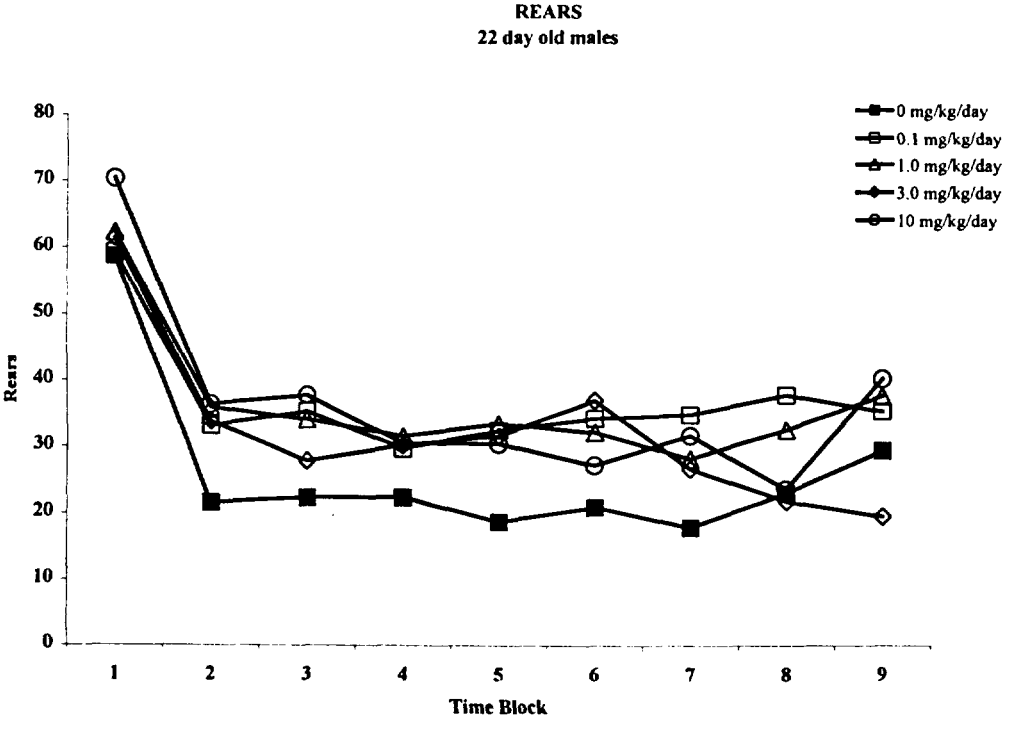
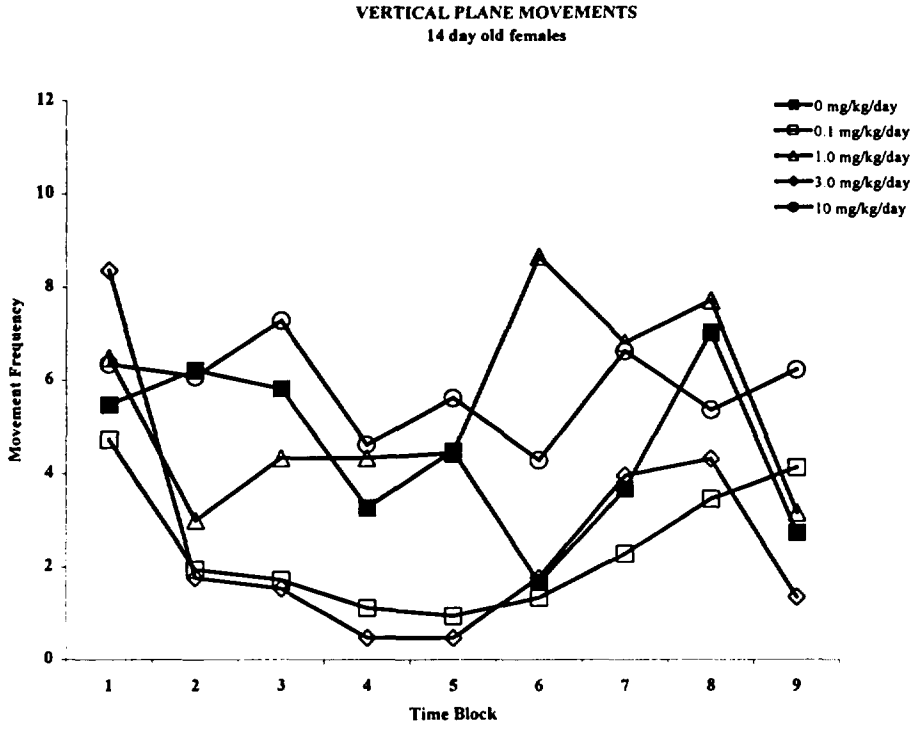

REARS
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Rears
Time Block
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9

Figure 22.


VERTICAL PLANE MOVEMENTS
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Movement Frequency
Time Block
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9

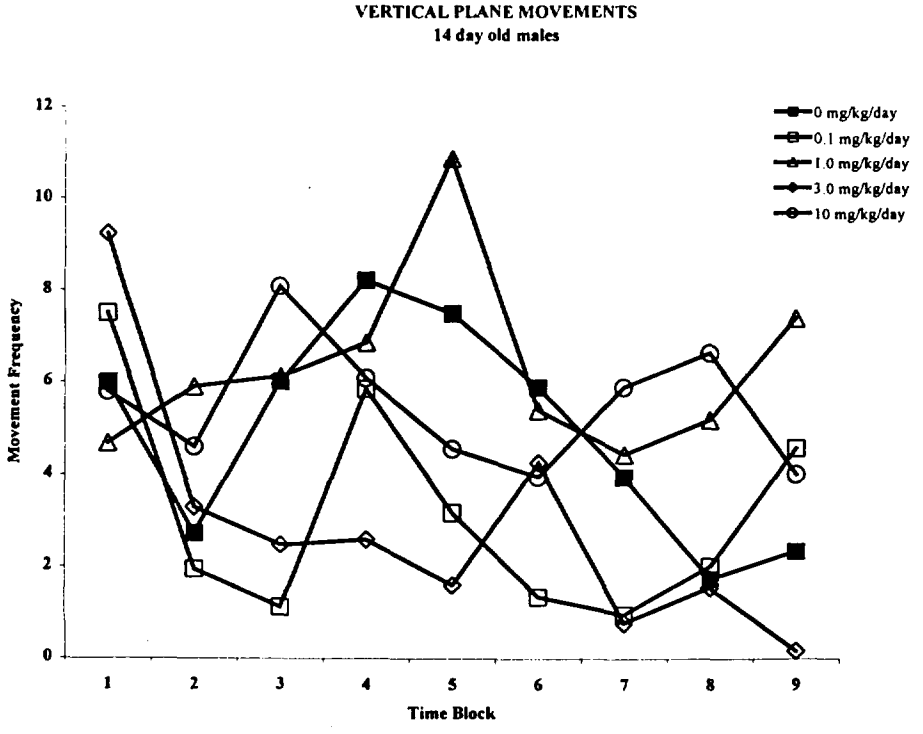
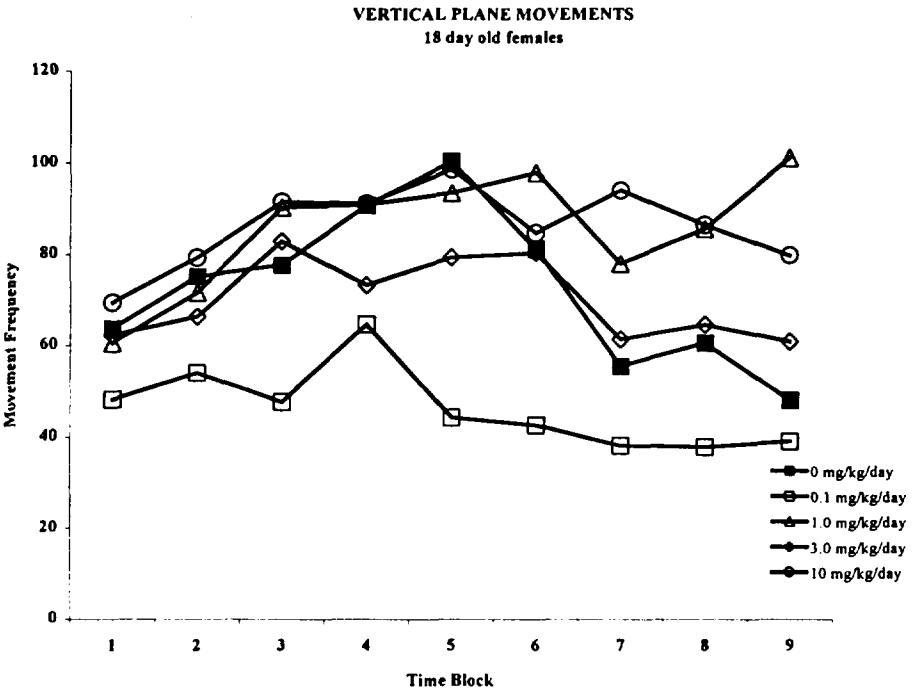

VERTICAL PLANE MOVEMENTS
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Movement Frequency
Time Block
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9

Figure 23.


VERTICAL PLANE MOVEMENTS
18 day old females
Movement Frequency
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

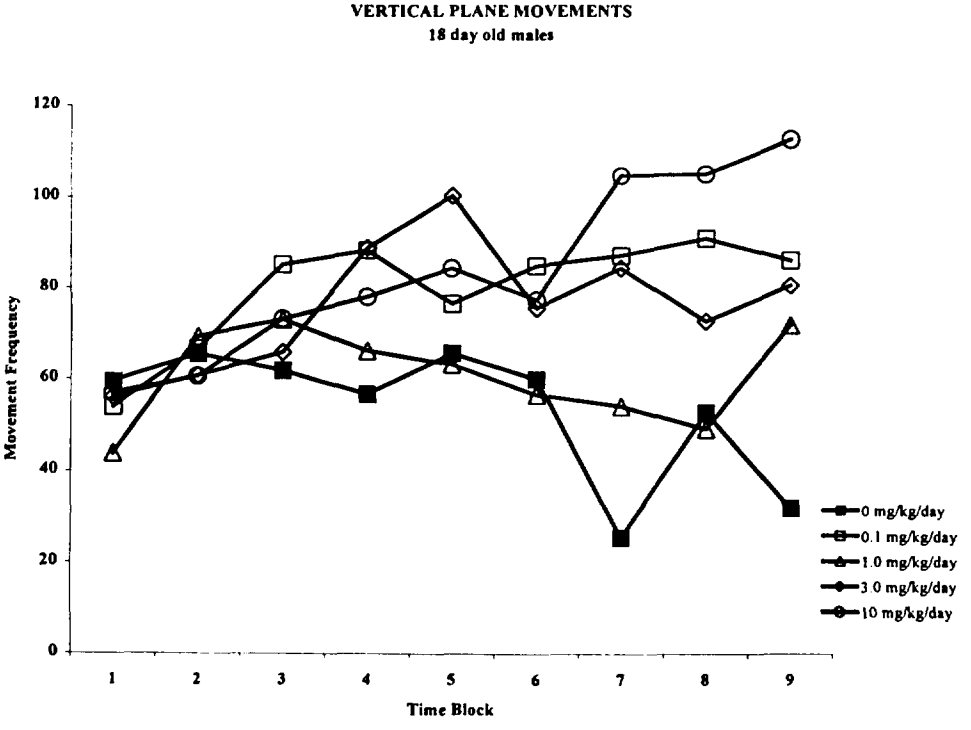
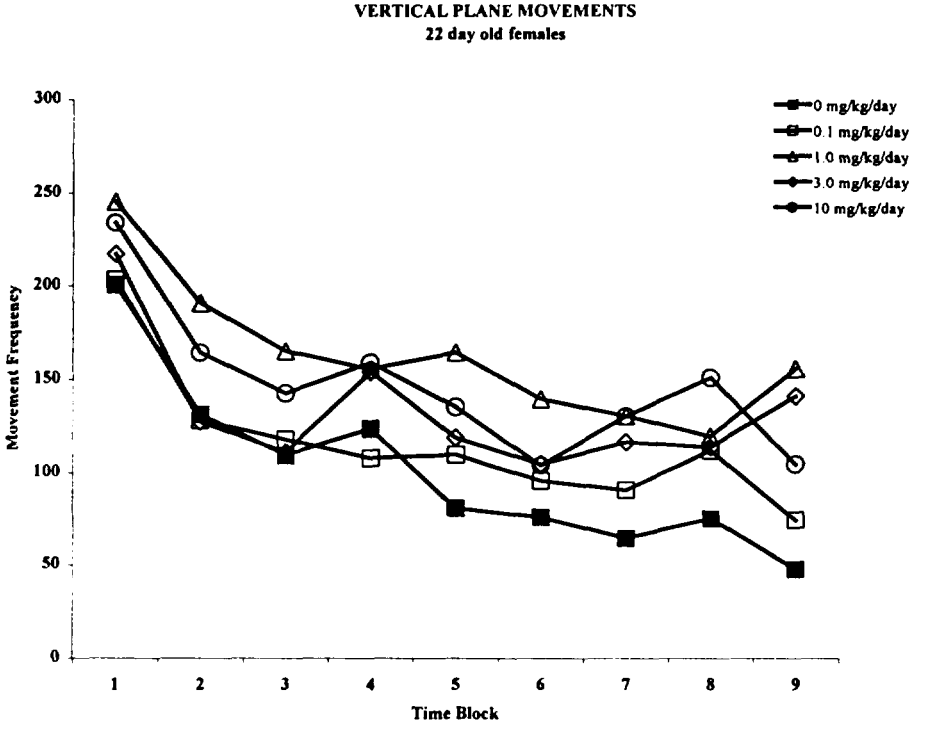

VERTICAL PLANE MOVEMENTS
18 day old males
Movement Frequency
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 24.


VERTICAL PLANE MOVEMENTS
22 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Movement Frequency
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

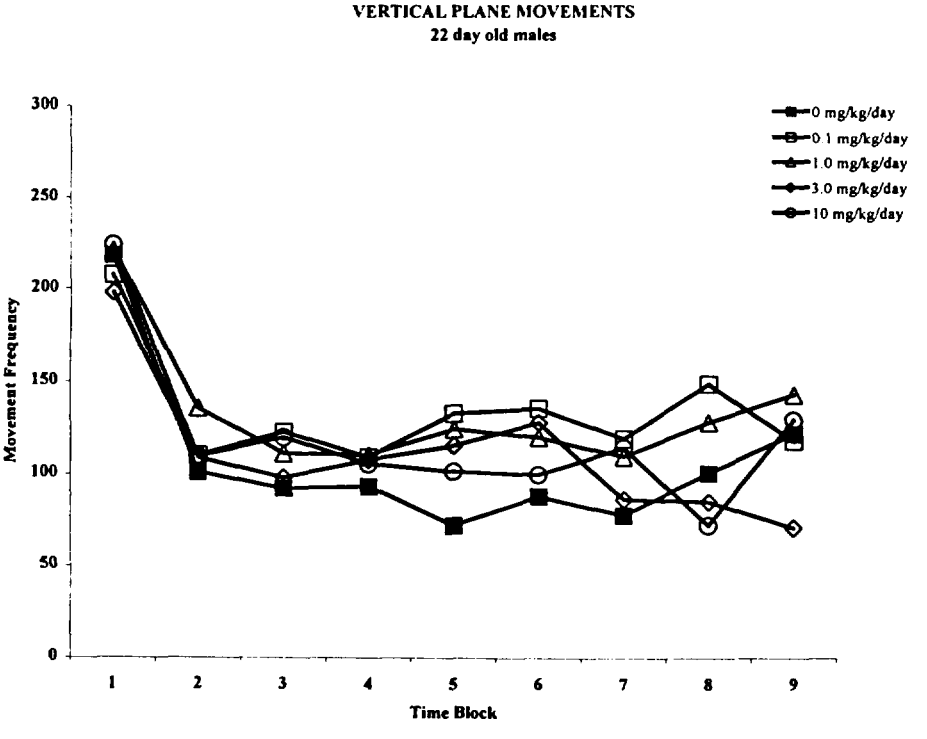
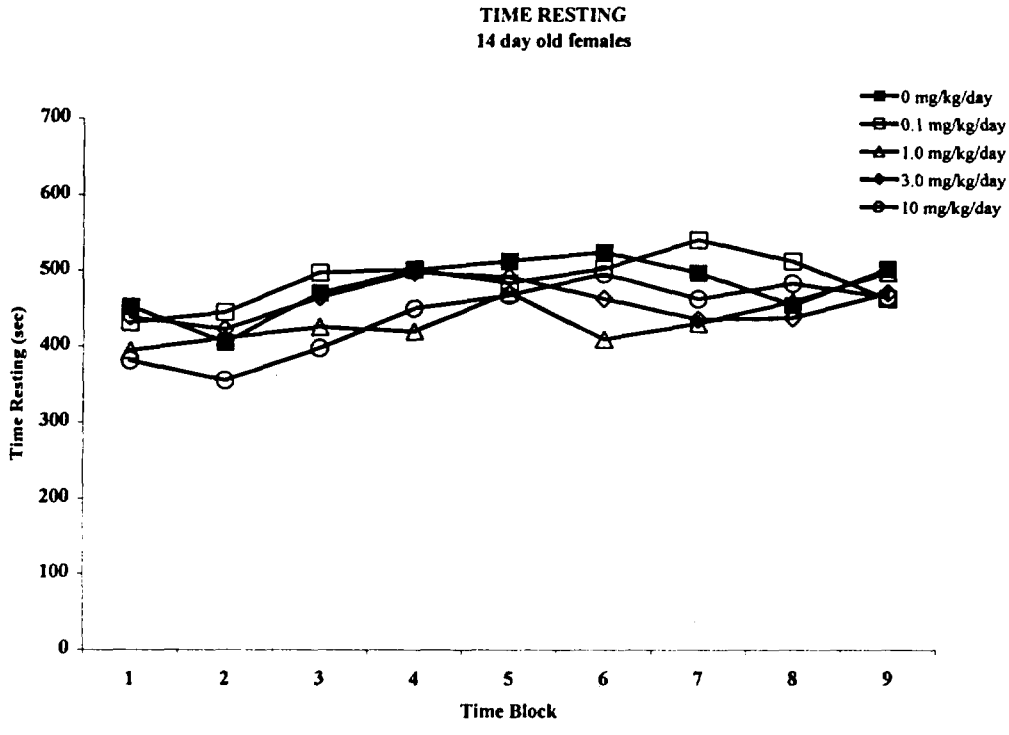

VERTICAL PLANE MOVEMENTS
22 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Movement Frequency
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 25.


TIME RESTING
14 day old females
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block

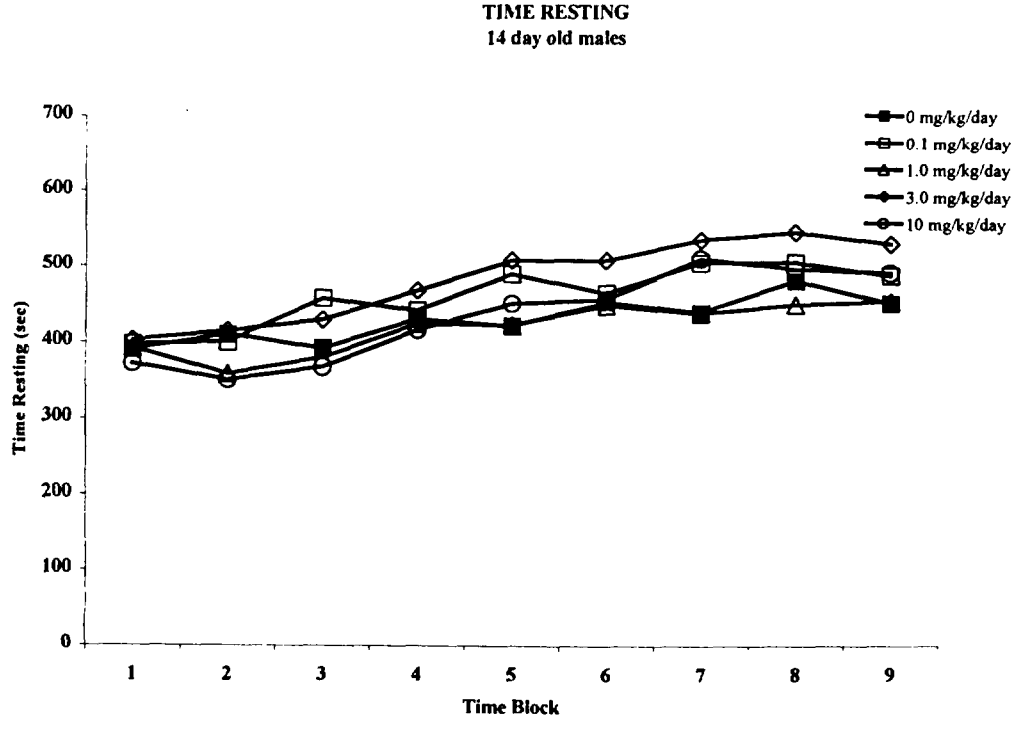
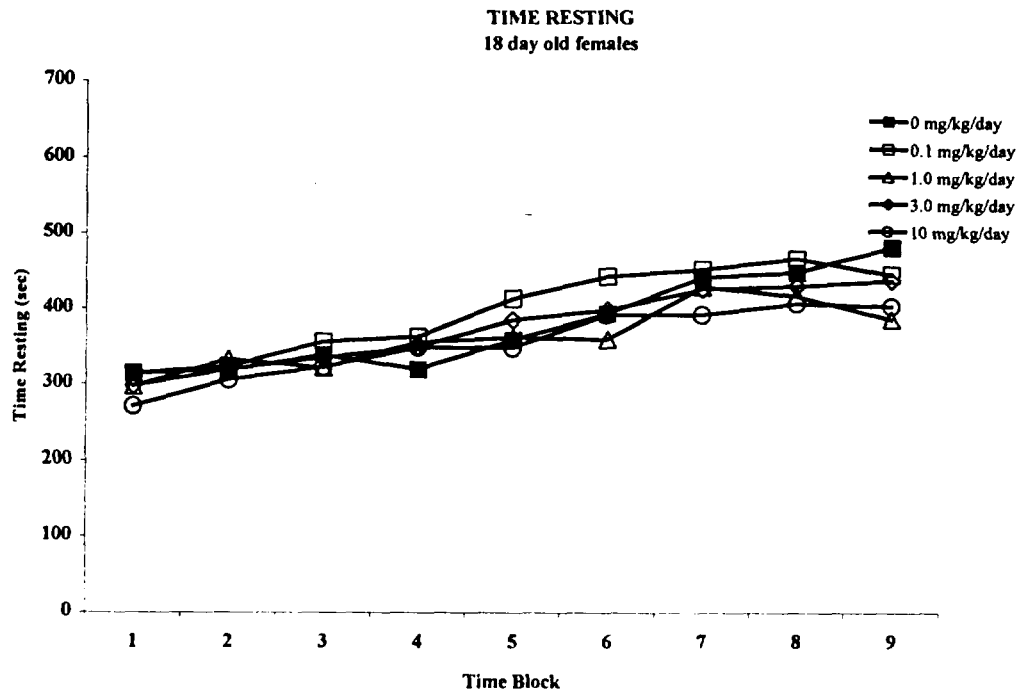

TIME RESTING
14 day old males
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block

Figure 26.


TIME RESTING
18 day old females
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

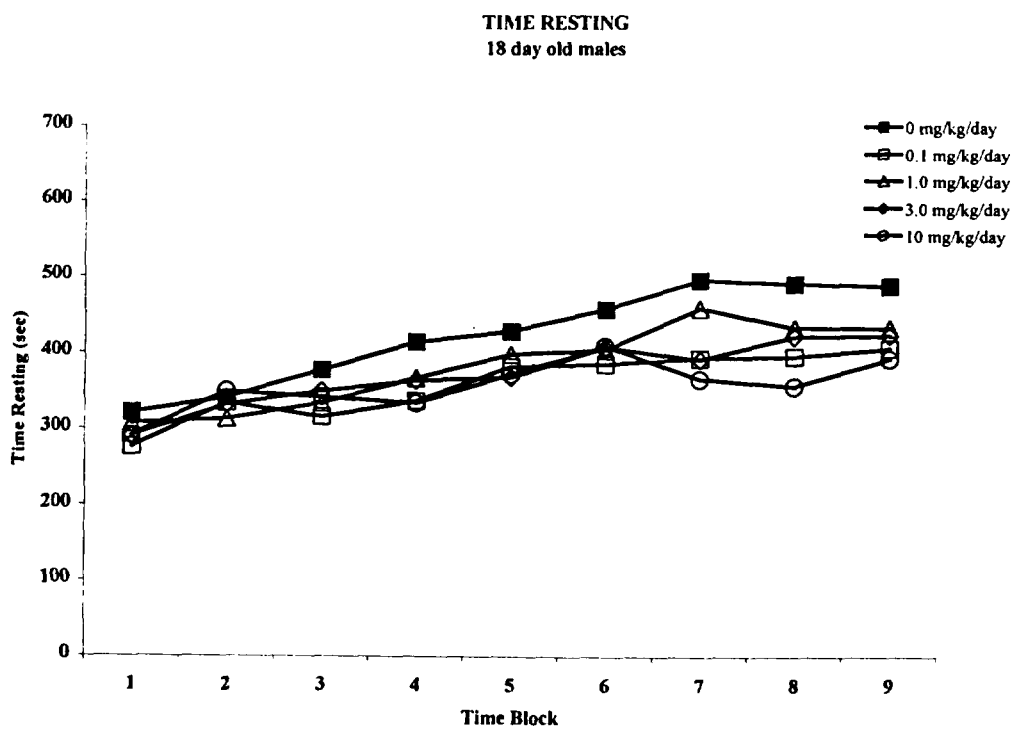
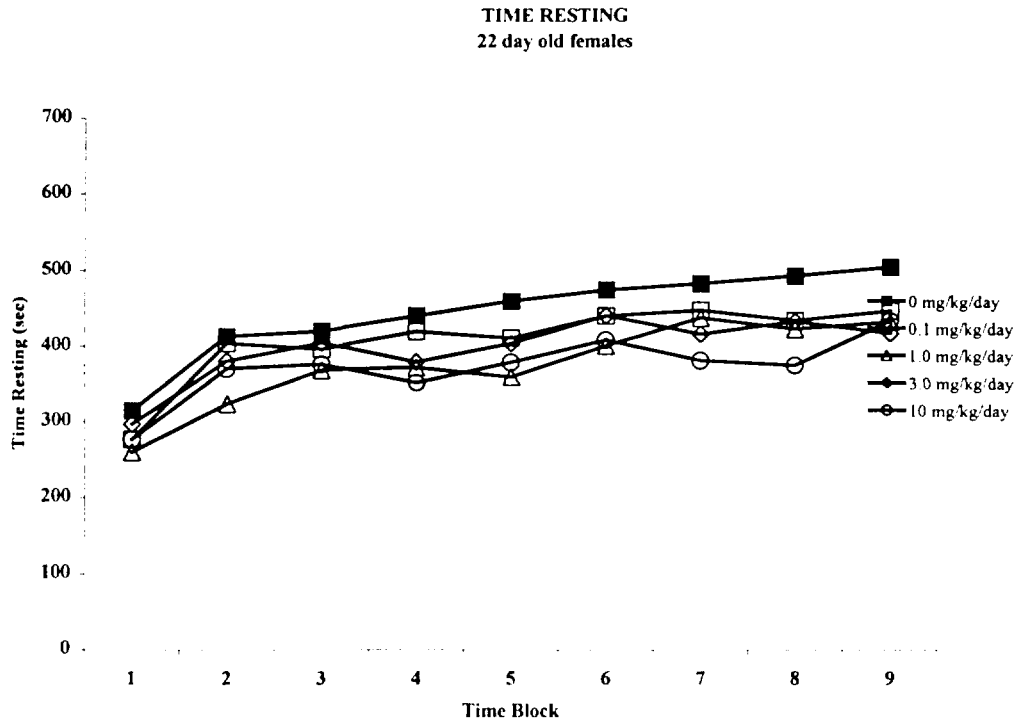

TIME RESTING
18 day old males
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Figure 27.


TIME RESTING
22 day old females
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

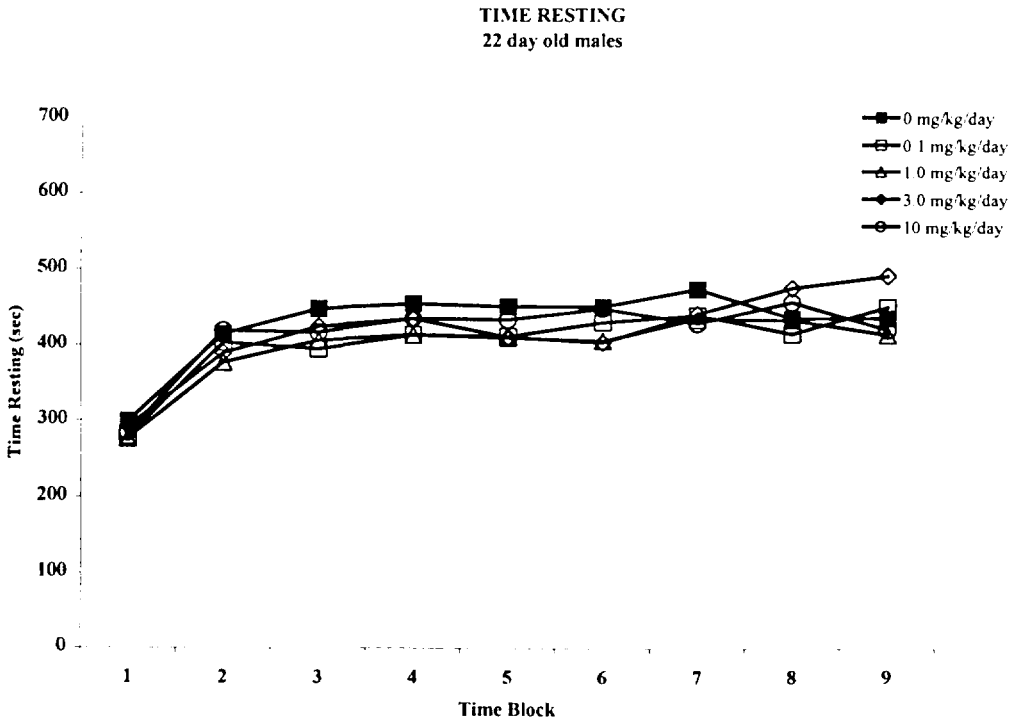

TIME RESTING
22 day old males
Time Resting (sec)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
Time Block
0 mg/kg/day
0.1 mg/kg/day
1.0 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day

Table 1.

MEAN DOSE AMMONIUM PERCHLORATE CONSUMED THROUGHOUT STUDY		
	MEAN	STD DEV
0.0 mg/kg/day	0.00	0.00
0.1 mg/kg/day	0.13	0.03
1.0 mg/kg/day	1.35	0.38
3.0 mg/kg/day	3.71	1.01
10.0 mg/kg/day	12.39	2.88

Table 2.

AMBULATORY MOVEMENTS		
	MEAN	STD DEV
PND14	477.6	688.4
PND18	967.5	789.7
PND22	760.6	596.5

TIME AMBULATORY		
	MEAN	STD DEV
PND14	35.0	45.3
PND18	57.1	41.2
PND22	43.0	30.7

STEREOTYPIC BURSTS		
	MEAN	STD DEV
PND14	343.5	324.8
PND18	499.9	302.2
PND22	420.8	252.8

STEREOTYPIC TIME		
	MEAN	STD DEV
PND14	113.8	87.5
PND18	167.6	94.7
PND22	152.9	86.1

HORIZONTAL MOVEMENTS		
	MEAN	STD DEV
PND14	787.4	959.9
PND18	1460.3	1094.9
PND22	1197.4	851.0

DISTANCE TRAVELED		
	MEAN	STD DEV
PND14	726.4	1000.6
PND18	1229.3	933.3
PND22	919.9	683.3

REARS		
	MEAN	STD DEV
PND14	1.9	3.8
PND18	27.4	27.5
PND22	34.4	33.1

VERTICAL PLANE MOVEMENTS		
	MEAN	STD DEV
PND14	4.3	9.1
PND18	71.1	71.8
PND22	127.1	113.1

TIME RESTING		
	MEAN	STD DEV
PND14	451.2	128.2
PND18	375.3	133.7
PND22	404.1	115.2

Table 3.

AMBULATORY MOVEMENTS			TIME AMBULATORY	
	MEAN	STD DEV	MEAN	STD DEV
0-10 min	1002.1	655.2	63.3	33.6
11-20 min	782.1	594.2	50.5	33.1
21-30 min	783.2	645.1	48.8	36.3
31-40 min	766.8	706.7	46.9	40.0
41-50 min	723.9	725.7	43.6	41.1
51-60 min	690.9	791.5	41.4	44.8
61-70 min	641.5	777.0	38.0	43.2
71-80 min	620.3	757.0	37.0	42.4
81-90 min	606.2	764.1	35.8	41.8

STEREOTYPIC BURSTS			STEREOTYPIC TIME	
	MEAN	STD DEV	MEAN	STD DEV
0-10 min	607.5	224.0	207.5	63.7
11-20 min	513.9	254.4	179.2	75.8
21-30 min	471.8	279.9	161.2	84.5
31-40 min	434.3	298.4	147.5	89.5
41-50 min	397.9	301.2	136.0	92.8
51-60 min	367.8	315.4	125.4	95.3
61-70 min	337.4	307.2	116.2	93.6
71-80 min	335.0	304.1	115.7	93.3
81-90 min	327.0	296.1	114.1	90.7

HORIZONTAL MOVEMENTS			DISTANCE TRAVELED	
	MEAN	STD DEV	MEAN	STD DEV
0-10 min	1574.1	888.3	1313.0	754.8
11-20 min	1268.2	825.1	1041.0	731.6
21-30 min	1232.3	903.4	1025.5	801.3
31-40 min	1192.2	990.2	998.5	888.8
41-50 min	1115.4	1015.6	936.6	921.2
51-60 min	1060.7	1100.2	896.3	1010.6
61-70 min	988.6	1082.8	829.3	979.5
71-80 min	961.9	1058.7	806.6	962.6
81-90 min	941.9	1057.0	780.0	951.6

Table 3 continued

	REARS		VERTICAL PLANE MOVEMENTS	
	MEAN	STD DEV	MEAN	STD DEV
0-10 min	29.9	34.2	93.4	109.7
11-20 min	22.4	27.4	67.3	87.0
21-30 min	21.8	27.4	66.2	85.2
31-40 min	21.7	27.3	68.5	88.0
41-50 min	21.0	27.9	67.0	90.5
51-60 min	19.1	27.4	62.6	89.4
61-70 min	17.6	26.8	58.7	88.0
71-80 min	18.6	27.5	61.9	91.4
81-90 min	18.9	29.0	62.2	96.0

	TIME RESTING	
	MEAN	STD DEV
0-10 min	329.2	94.9
11-20 min	370.3	106.1
21-30 min	390.0	118.4
31-40 min	405.7	127.0
41-50 min	420.4	130.9
51-60 min	433.2	136.5
61-70 min	445.8	133.8
71-80 min	447.3	132.4
81-90 min	450.0	129.4

Table 4.

AMBULATORY MOVEMENTS						
	14 days		18 days		22 days	
	MEAN	STD DEV	MEAN	STD DEV	MEAN	STD DEV
0-10 min	409.5	379.4	1186.6	607.4	1410.1	474.5
11-20 min	541.4	544.2	1069.1	595.2	735.9	519.5
21-30 min	541.3	623.0	1081.9	662.1	726.5	524.5
31-40 min	520.4	725.4	1043.6	708.8	736.5	580.0
41-50 min	460.0	713.9	972.7	788.4	739.0	567.8
51-60 min	525.6	848.9	890.0	871.8	657.3	581.1
61-70 min	466.7	774.0	829.8	906.8	628.0	574.7
71-80 min	427.8	744.4	811.1	880.1	621.8	568.5
81-90 min	406.0	724.5	822.3	922.4	590.2	543.3
TIME AMBULATORY						
0-10 min	34.6	27.3	76.9	30.9	78.5	21.1
11-20 min	42.6	36.6	65.6	30.1	43.3	26.5
21-30 min	40.1	41.4	64.5	33.7	41.7	27.2
31-40 min	37.2	47.6	61.8	36.6	41.7	29.5
41-50 min	32.8	47.0	56.3	41.6	41.7	29.4
51-60 min	36.2	54.3	50.9	45.0	37.1	30.4
61-70 min	32.4	50.2	46.4	46.0	35.2	29.3
71-80 min	30.5	49.4	45.5	44.6	35.1	29.4
81-90 min	28.7	47.0	45.7	46.5	33.2	27.1
HORIZONTAL MOVEMENTS						
0-10 min	770.8	545.2	1822.4	812.8	2129.0	630.8
11-20 min	927.2	768.1	1659.0	809.7	1218.4	729.2
21-30 min	889.1	884.5	1644.5	902.6	1163.5	753.1
31-40 min	833.9	1012.8	1584.8	983.4	1157.9	821.6
41-50 min	742.1	999.9	1449.9	1096.1	1154.1	806.9
51-60 min	815.0	1164.2	1328.5	1206.9	1038.7	836.7
61-70 min	744.3	1073.5	1227.4	1251.1	994.2	834.3
71-80 min	691.7	1040.7	1208.5	1219.1	985.5	819.6
81-90 min	672.3	995.4	1217.8	1273.6	935.6	776.4

Table 4 continued

DISTANCE TRAVELED						
	14 days		18 days		22 days	
	MEAN	STD DEV	MEAN	STD DEV	MEAN	STD DEV
0-10 min	653.4	567.0	1609.2	699.9	1676.4	496.0
11-20 min	848.3	788.5	1375.4	685.5	899.3	592.2
21-30 min	824.2	899.7	1368.7	762.7	883.6	602.9
31-40 min	783.4	1045.8	1319.2	826.7	892.8	663.8
41-50 min	693.9	1046.1	1220.2	940.1	895.7	660.0
51-60 min	783.7	1227.2	1112.3	1024.9	792.9	670.2
61-70 min	698.0	1122.2	1027.5	1064.5	762.3	660.2
71-80 min	647.9	1098.4	1011.7	1035.7	760.2	665.0
81-90 min	604.7	1042.6	1019.7	1083.7	715.7	611.0
STEREOTYPIC BURSTS						
0-10 min	443.8	234.6	695.4	191.5	683.5	137.6
11-20 min	480.0	299.0	612.1	206.8	449.4	218.4
21-30 min	408.9	320.9	585.5	243.8	421.1	232.3
31-40 min	344.8	341.0	547.6	265.0	410.7	245.2
41-50 min	297.2	318.7	485.0	307.4	411.6	243.3
51-60 min	299.5	346.3	432.7	321.5	371.1	259.7
61-70 min	276.5	330.4	385.1	326.0	350.5	250.1
71-80 min	272.9	334.1	380.3	314.4	351.9	248.8
81-90 min	267.7	309.7	375.7	324.9	337.4	237.9
STEREOTYPIC TIME						
0-10 min	160.3	65.1	225.2	52.9	237.0	41.8
11-20 min	160.0	81.3	208.1	62.0	169.6	74.4
21-30 min	131.7	85.9	197.4	73.9	154.5	80.0
31-40 min	109.5	87.4	183.8	82.8	149.0	82.9
41-50 min	96.9	85.8	162.8	97.2	148.4	81.9
51-60 min	94.4	87.5	146.2	101.2	135.5	89.2
61-70 min	90.5	84.7	129.9	103.2	128.3	87.1
71-80 min	88.9	87.4	128.7	99.3	129.4	87.4
81-90 min	91.8	80.9	126.4	103.2	124.2	82.8

Table 4 continued

	14 days		18 days		22 days	
REARS						
	MEAN	STD DEV	MEAN	STD DEV	MEAN	STD DEV
0-10 min	2.4	3.3	25.4	20.4	62.0	35.8
11-20 min	1.6	2.7	28.8	22.5	36.8	32.7
21-30 min	1.9	3.5	30.4	26.1	33.3	31.0
31-40 min	1.8	3.6	31.0	26.6	32.3	30.3
41-50 min	1.9	4.1	30.2	29.2	30.9	30.5
51-60 min	2.0	4.3	26.7	29.6	28.5	30.5
61-70 min	1.7	4.1	23.6	29.4	27.6	29.8
71-80 min	1.9	4.4	25.2	30.2	28.7	30.4
81-90 min	1.6	3.9	25.6	31.3	29.6	32.9
VERTICAL PLANE MOVEMENTS						
0-10 min	6.5	10.0	56.9	45.7	217.0	98.6
11-20 min	3.7	6.6	66.7	52.0	131.4	108.8
21-30 min	4.3	8.3	74.8	63.1	119.4	105.2
31-40 min	4.3	8.5	78.7	65.5	122.4	108.5
41-50 min	4.3	9.7	79.8	76.2	116.8	110.2
51-60 min	3.9	8.7	73.8	80.0	110.1	108.3
61-70 min	3.9	9.7	67.9	83.3	104.4	105.3
71-80 min	4.5	9.7	70.0	83.2	111.3	111.0
81-90 min	3.6	9.5	71.5	84.7	111.5	120.5
TIME RESTING						
0-10 min	405.1	89.6	297.9	81.5	284.6	60.4
11-20 min	397.4	114.6	326.2	89.6	387.1	98.9
21-30 min	428.2	124.2	338.1	105.7	403.8	106.0
31-40 min	453.3	132.4	354.4	117.5	409.3	111.0
41-50 min	470.3	128.7	380.9	137.2	409.9	109.7
51-60 min	469.4	137.9	402.9	144.3	427.4	118.3
61-70 min	477.1	131.6	423.7	147.5	436.5	115.1
71-80 min	480.6	132.7	425.7	142.1	435.5	115.0
81-90 min	479.5	124.2	427.9	147.7	442.7	108.7

QUALITY ASSURANCE STATEMENT

A NEURDEVELOPMENTAL STUDY INTO THE EFFECTS OF ORAL AMMONIUM PERCHOLATRE EXPOSURE ON THE MOTOR ACTIVITY OF PRE-WEANLING RAT PUPS

The conduct of this study has been subjected to periodic inspections of critical phases by the Naval Health Research Center Toxicology Detachment (NHRC/TD) Assurance Unit.

The dates of inspection and the critical phase are given below: Dosing was initiated on February 8, 2000.

Dates of QA Inspection	*Critical Phase*
December 15-16, 1999 April 20, 2000	Perchlorate Team GLP Training
February 29, 2000	Receipt, quarantine of rats Protocol approval Weeks 1 and 2 data entrees Animal ID system Randomization Procedures
March 7, 2000	Facilities and animal care support Notebook entrees for Week 3 Balance check weights Mating and randomization of breeding pairs Dose concentration verification
March 21, 2000	Notebook entrees for Weeks 4 and 5 Light cycle control Pregnancy records
March 31, 2000	Team understanding of protocol requirements Breeding records and performance Notebook entrees for Weeks 5 and 6
April 27, 2000	Pup culling Mating records Notebook entrees for the balance of the Whole life portion of the study OPTIVERIMIX S.O.P. and data collection Daily room records for animal care service Mating records

These critical phases of the Neurodevelopmental study with Ammonium Perchlorate have been inspected on the dates indicated. The NHRC/TD Quality Assurance Unit conducted these inspections. No deviations from the Protocol and approved amendments or the Standard Operating Procedures were noted that effected the quality and integrity of the study.

A data audit was performed by the QAU on June 22-23, 2000.

This Quality Assurance Statement accurately describes the inspections of critical phases and Standard Operating Procedures and data entrees and the formal report audit for the Neurodevelopmental Study with Ammonium Perchlorate in rats performed at NHRC/TD.

Individual pup data and average for the six end points were audited and no errors were detected in the data entrees. Average data for pup end points were accurately transferred to data tables. Statistical review was consistent with the trends seen on the visual audit.

Signed _____ Date:_____

Head, NHRC/TD Quality Assurance Unit